=> fil ca,caplus; d que l21
FILE 'CA' ENTERED AT 15:33:19 ON 02 APR 96
USE IS SUBJECT TO THE TERMS OF YOUR CUSTOMER AGREEMENT
COPYRIGHT (C) 1996 AMERICAN CHEMICAL SOCIETY (ACS)

FILE 'CAPLUS' ENTERED AT 15:33:19 ON 02 APR 96
USE IS SUBJECT TO THE TERMS OF YOUR CUSTOMER AGREEMENT
COPYRIGHT (C) 1996 AMERICAN CHEMICAL SOCIETY (ACS)

-Key Terms

L13 83 SEA FILE=CA ((BISPECIF? OR BI SPECIF? OR HYBRID)(W)ANTIBO D? OR HETEROBIFUNCT? OR HETERO(W)(BIFUNCT? OR BI FUNCT? O R CONJUGAT?) OR HETEROCONJUG? OR DUAL? SPECIF? OR QUADROM ?)(L)(FC? OR CD16 OR CD 16)
L14 83 SEA FILE=CAPLUS ((BISPECIF? OR BI SPECIF? OR HYBRID)(W)AN TIBOD? OR HETEROBIFUNCT? OR HETERO(W)(BIFUNCT? OR BI FUNC T? OR CONJUGAT?) OR HETEROCONJUG? OR DUAL? SPECIF? OR QUA DROM?)(L)(FC? OR CD16 OR CD 16)
L15 166 SEA ((BISPECIF? OR BI SPECIF? OR HYBRID)(W) ANTIBOD? OR H ETEROBIFUNCT? OR HETERO(W)(BIFUNCT? OR BI FUNCT? OR CONJU GAT?) OR HETEROCONJUG? OR DUAL? SPECIF? OR QUADROM?)(L)(F C? OR CD16 OR CD 16)
L16 70 SEA FILE=CA RAN=(,1994) ((BISPECIF? OR BI SPECIF? OR HYBR ID)(W)ANTIBOD? OR HETEROBIFUNCT? OR HETERO(W)(BIFUNCT? OR BI FUNCT? OR CONJUGAT?) OR HETEROCONJUG? OR DUAL? SPECIF ? OR QUADROM?)(L)(FC? OR CD16 OR CD 16)
L17 70 SEA FILE=CAPLUS RAN=(,1994) ((BISPECIF? OR BI SPECIF? OR HYBRID)(W)ANTIBOD? OR HETEROBIFUNCT? OR HETERO(W)(BIFUNCT ? OR BI FUNCT? OR CONJUGAT?) OR HETEROCONJUG? OR DUAL? SP ECIF? OR QUADROM?)(L)(FC? OR CD16 OR CD 16)
L18 140 SEA L15
L19 39 SEA FILE=CA L16(L)ANTIGEN?
L20 39 SEA FILE=CAPLUS L17(L)ANTIGEN?
L21 78 SEA L18(L) ANTIGEN?

=> dup rem l21; d 1-39 .beverly; fil
biosi,medl,embas,lifesci,cancerlit,biotechds,wpids,dissabs,confsci,scisearc
h
PROCESSING COMPLETED FOR L21
L22 39 DUP REM L21 (39 DUPLICATES REMOVED)

L22 ANSWER 1 OF 39 CA COPYRIGHT 1996 ACS DUPLICATE 1
AN 121:106521 CA
TI Oligomeric, multispecific derivatives of immunoglobins and their preparation and use
SO PCT Int. Appl., 179 pp.
CODEN: PIXXD2
IN Holliger, Kaspar-Philipp; Griffiths, Andrew David; Hoogenboom, Hendricus Renerus J.; Malmqvist, Magnus; Marks, James David; McGuinness, Brian Timothy; Pope, Anthony Richard; Prospero, Terence Derek; Winter, Gregory Paul

AI WO 93-GB2492 931203
PI WO 9413804 A1 940623
PY 1994
AB Proteins with domains derived from the binding region of an Ig heavy chain variable region and a binding region of an Ig light chain variable region that are linked but prevented from interacting to form an antigen binding site, assoc. to form antigen binding multimers, such as dimers, which may be multivalent or have multiple specificities. The domains may be linked by a short peptide linker or may be joined directly together with bispecific dimers linked by longer linkers. Methods of prepn. of the polypeptides and multimers and of diverse repertoires of such proteins and their display on the surface of bacteriophage for easy selection of binders of interest, are described. along with many utilities. The proteins have many anal., diagnostic, and therapeutic uses. Modeling of Igs indicated that directly joining the VH antigen-binding domain to the VL antigen-binding domain would be possible with some structural constraints on higher-order structures but without the interaction of the two domains and so allow the generation of single-chain, bispecific antibodies (diabodies). The invention is demonstrated by construction and characterization of a no. of diabodies. The genes for these antibodies were constructed by std. methods and expressed in Escherichia coli.

L22 ANSWER 2 OF 39 CA COPYRIGHT 1996 ACS DUPLICATE 2
AN 120:215158 CA
TI Targeting HIV-1 to Fc.gamma.R on human phagocytes via bispecific antibodies reduces infectivity of HIV-1 to T cells
SO J. Leukocyte Biol. (1994), 55(3), 385-91
CODEN: JLBIE7; ISSN: 0741-5400
AU Howell, Alexandra L.; Guyre, Paul M.; You, Kwansa; Fanger, Michael W.
PY 1994
AB In addn. to CD4, the primary receptor to which the human immunodeficiency virus type 1 (HIV-1) binds, mononuclear phagocytes (monocytes) express 3 classes of **Fc** receptors for IgG (**Fc**.gamma.R). The authors have previously shown that infection of monocytes by HIV-1 is inhibited when **bispecific antibodies** (BsAbs) are used to target the virus to either the type I, type II, or type III **Fc**.gamma.R on these cells. Infection of monocytes was not inhibited when HIV-1 was targeted to either human leukocyte **antigen** class I or CD33. The authors have extended these studies to examine the ability of BsAbs plus polymorphonuclear leukocytes (neutrophils, PMNs) and monocytes to reduce infectivity of HIV-1 to cells from the human T cell lymphoma line, H9. The prodn. of HIV-1 following interaction of virus with BsAb and phagocytes was detd. in an indicator cell assay by mixing BsAb, HIV-1, and phagocytes with uninfected H9 cells. Productive infection of H9 cells was quantitated on subsequent days by measuring p24 gag **antigen** levels in supernatants by ELISA. Evidently the addn. of interferon-.gamma.-activated PMNs or monocytes to cultures of HIV-1 plus H9 cells in the absence of BsAb results in a marked redn. in

p24 levels equiv. to 85-90% of control levels. With the combination of BsAb (anti-**Fc**.gamma.RI .times. anti-gp120) plus IFN-.gamma.-activated phagocytes, levels of p24 in H9 cultures were below those at culture initiation. Thus, IFN-.gamma.-activated phagocytes can affect the natural course of HIV-1 infection of T cells, a finding of potential clin. importance.

L22 ANSWER 3 OF 39 CA COPYRIGHT 1996 ACS DUPLICATE 3
AN 120:242115 CA
TI A bifunctional murine::human chimeric antibody with one antigen-binding arm replaced by bacterial .beta.-lactamase
SO Mol. Immunol. (1994), 31(4), 261-7
CODEN: MOIMD5; ISSN: 0161-5890
AU De Sutter, Kristine; Fiers, Walter
PY 1994
AB The authors genetically engineered a murine::human chimeric antibody-directed against the tumor marker human placental alk. phosphatase, in which one **antigen**-binding arm (Fab) has been replaced by Escherichia coli .beta.-lactamase (Bla). A mutated Bla gene in which the termination codon had been replaced by GAG, was fused in-phase to the cDNA sequence encoding the hinge region, CH2 and CH3 of the human IgG3 heavy chain. The resulting BlaHG3f fusion gene was placed under control of the Simian Virus 40 late promoter, and transiently expressed in COS-1 cells together with the genes encoding the murine light and murine::human chimeric heavy chains. Approx. 200 ng/mL of correctly assembled bifunctional antibody-Bla immunoconjugates were detected in the culture supernatant. This observation indicates that Bla (with its own leader peptide) can efficiently direct secretion into the culture medium of adventitious sequences fused at its C-terminus. Furthermore, the assembly in the **Fc** region was not affected by steric hindrance due to a Bla moiety and an Fab arm in close proximity. The antibody-Bla immunoconjugate could be of therapeutic value for the activation of cephalosporin-based anti-cancer prodrugs at the tumor site. Moreover, the expression strategy adopted here is particularly suitable for a quick and convenient anal. of newly designed gene products in which the Bla moiety has been replaced by other enzymes or by **antigen** -binding fragments in order to engineer **bispecific antibodies**.

L22 ANSWER 4 OF 39 CA COPYRIGHT 1996 ACS DUPLICATE 4
AN 120:104487 CA
TI Promotion of natural killer cell growth in vitro by bispecific (anti-CD3 .times. anti-CD16) antibodies
SO Immunology (1994), 81(1), 92-5
CODEN: IMMUAM; ISSN: 0019-2805
AU Malygin, A. M.; Somersalo, K.; Timonen, T.
PY 1994
AB Bispecific heteroconjugated F(ab')2 fragments were prepd. from pepsin-digested monoclonal OKT3 (anti-CD3) and 3G8 (anti-CD16) antibodies with 5,5'-dithiobis-(2-nitrobenzoic acid). When these bispecific antibodies (BsA) were added to peripheral blood

lymphocyte (PBL) cultures with 100 U/mL human recombinant interleukin-2 (rIL-2), preferable growth of natural killer cells occurred. After 3 wk the frequencies of CD56+ and CD56+3- cells in cultures with BsA were 74% and 65%, resp., compared with 48% and 29% in control cultures. To det. the frequencies of CD3+ lymphocytes in the presence of BsA, cells from 1-day cultures were labeled with fluorescein isothiocyanate (FITC)-conjugated anti-CD3, CD4, and CD8 monoclonal antibodies (mAb) and propidium iodide which stains dead cells. Flow cytometry revealed that >95% of the dead cells in cultures with BsA were CD3+. Thirty-seven per cent of CD3+, 43% of CD4+, and 17% of CD8+ cells were dead on day 1, and after 3 days the CD4+/CD8+ ratio among viable lymphocytes was 1.6 in the control and 0.5 in BsA cultures. Thus, bispecific (anti-CD3 x anti-CD16) F(ab')2 fragments are strongly immunomodulatory by inducing the killing of T cells by CD16+ cells.

L22 ANSWER 5 OF 39 CA COPYRIGHT 1996 ACS DUPLICATE 5
AN 121:154910 CA
TI Production and use of anti-FcR bispecific antibodies
SO ImmunoMethods (1994), 4(1), 72-81
CODEN: IMUME8; ISSN: 1058-6687
AU Fanger, Michael W.; Graziano, Robert F.; Guyre, Paul M.
PY 1994
AB A review, with 91 refs. **Bispecific antibodies** (BsAb) are antibodies with two different specificities. BsAb composed of anti-**Fc**.gamma.R Ab linked to anti-target Ab have been useful in exploring the function of the three classes of human **Fc**.gamma.R. In addn., BsAb have been developed as new agents for immunotherapy which can join together different mols. or cells. In directed or redirected cytotoxicity, BsAb that bind both to target cells (pathogens or tumors) and to triggering mols. on leukocytes such as **Fc**.gamma.R are used to focus normal cellular immune defense mechanisms specifically to the tumor cell or infectious agent. Limited clin. trials have demonstrated little toxicity and promising responses. This ability to redirect normal cytotoxic mechanisms to kill tumors, infectious agents, or infected cells makes BsAb powerful new therapeutic tools. In addn., BsAb are being used to target other appropriate mols. to **Fc** .gamma.R, including **antigens** as vaccine adjuvants and immune complexes. This review focuses on BsAb in which one specificity is directed to **Fc**.gamma.R on human leukocytes.

L22 ANSWER 6 OF 39 CA COPYRIGHT 1996 ACS DUPLICATE 6
AN 119:223796 CA
TI Involvement of the high-affinity receptor for IgG (Fc.gamma.RI; CD64) in enhanced tumor cell cytotoxicity of neutrophils during granulocyte colony-stimulating factor therapy
SO Blood (1993), 82(3), 931-9
CODEN: BLOOAW; ISSN: 0006-4971
AU Valerius, Thomas; Repp, Roland; de Wit, Ton P. M.; Berthold, Susanne; Platzer, Erich; Kalden, Joachim R.; Gramatzki, Martin; van de Winkel, Jan G. J.
PY 1993

...gamma.R). The authors have previously shown that infection of monocytes by HIV-1 is inhibited when **bispecific antibodies** (BsAbs) are used to target the virus to either the type I, type II, or type III **Fc**.gamma.R on these cells. Infection of monocytes was not inhibited when HIV-1 was

AB Three different classes of **Fc** receptors for IgG (**Fc**.gamma.R) are currently distinguished in humans, of which polymorphonuclear phagocytes (PMN) normally express both low-affinity receptor classes-**Fc**.gamma.RII (CD32) and **Fc**.gamma.RIII (**CD16**). During therapy with granulocyte colony-stimulating factor (G-CSF), neutrophils from patients with various malignancies and different hematol. disorders were found to addnl. express high levels of the receptor with high affinity for IgG (**Fc**.gamma.RI; CD64). For these patients, the relative fluorescence intensity (rFI) for **Fc**.gamma.RI was 5.3, compared with 1.0 for healthy donors. The expression of **Fc**.gamma.RI during G-CSF therapy could be confirmed by using a panel of 6 CD64-specific antibodies, and by showing mRNA for **Fc**.gamma.RI. So far, 3 genes for **Fc**.gamma.RI have been identified, encoding 4 distinct transcription products. By reverse transcriptase-polymerase chain reaction. technol., transcripts for both membrane-assocd. isoforms (hFc.gamma.RIa and hFc.gamma.RIb2) could be detected. The functional activity of **Fc**.gamma.RI on PMN during G-CSF therapy was shown by measuring binding of monomeric human IgG and antibody-dependent cellular cytotoxicity (ADCC). Thus, **Fc**.gamma.RI-pos. neutrophils displayed enhanced ADCC activity to glioma (A1207), squamous cell (A431), and ovarian (SK-ov3) carcinoma cell lines. The involvement of **Fc**.gamma.RI in this increased cytotoxic activity was shown by blocking **Fc**.gamma. receptors with monoclonal antibodies, and by using F(ab')2 .times. F(ab')2-**bispecific antibodies** with specificities against tumor-related **antigens** and **Fc**.gamma.RI, resulting in solely **Fc**.gamma.RI-mediated cytotoxicity. Therapeutically, this addnl. **Fc** receptor on PMN may increase the efficacy of exptl. antibody therapy.

L22 ANSWER 7 OF 39 CA COPYRIGHT 1996 ACS DUPLICATE 7
AN 119:70204 CA
TI Murine bispecific monoclonal antibodies as immunomodulators
SO Monoclonal Antibodies 2 (1993), 493-501. Editor(s): Epenetos, Agamemnon A. Publisher: Chapman and Hall, London, Uk. CODEN: 59CLAZ
AU DeMonte, L. B.; Momo, M.; Nistico, P.; Anachini, A.; Cavallo, F.; Mariani, M.; Knapp, W.; Letarte, M.; Natali, P. G.; Malavasi, F.
PY 1993
AB **Bispecific antibodies** featuring two distinct binding activities, have potential applications either for improving immunoassays or for delivering toxins, radionuclides and cytotoxic drugs to specific cell targets or organs. Nevertheless, their most promising immunotherapeutic application has been the retargeting of different subsets of cytotoxic cell effectors to selected tumors. To this aim the authors generated a panel of murine bispecific monoclonal antibodies (bsmAbs) by fusing established hybridomas previously made resistant to different metabolic drugs by means of retroviral shuttle vectors carrying the relevant resistance genes. The surface leukocyte mols. chosen were CD3, CD2, **CD16** and CD38, and the target mols. on the tumor side included the

high-mol.-wt. melanoma-assocd. **antigen** (HMW-MAA), the carcinoembryonic **antigen** (CEA) and the common acute lymphoblastic leukemia **antigen** (CALLA). Tissue uptake of the 125I-labeled bs-mAb was analyzed in a nude mouse model. The HMW-MAA+ Colo 38 melanoma cell line was grown in nude mice as established s.c. tumors and the tumor regression was quantitated after bs-mAb treatment.

L22 ANSWER 8 OF 39 CA COPYRIGHT 1996 ACS DUPLICATE 8
AN 119:247564 CA
TI Use of anti-CD3 and anti-CD16 bispecific monoclonl antibodies for the targeting of T and NK cells against tumor cells
SO Cancer Detect. Prev. (1993), 17(2), 295-30
CODEN: CDPRD4; ISSN: 0361-090X
AU Ferrini, Silvano; Cambiaggi, Anna; Sforzini, Sabrina; Canevari, Silvana; Mezzanzanica, Delia; Colnaghi, Maria Ines; Moretta, Lorenzo
PY 1993
AB To target T lymphocytes against EGF-R+ tumors, the authors constructed anti-CD3/anti-EGF-R bimAbs either by the generation of a hybrid hybridoma (quadroma) or by a chem. crosslinking method. Anal. of the in vitro functional activity of these two different constructs indicated that the quadroma-secreted bimAb was more efficient in targeting the CD3+8+ clones against EGF-R+ target cells with respect to the bimAb produced by chem. method. In addn., the quadroma-produced bimAb is able to induce cytolysis of EGF-R+ tumor cell lines of PHA-induced lymphoblasts that had been expanded in IL-2-contg. medium, whereas tumor cells lacking expression of EGF-R were not lysed. An anti-CD16 hybridoma (IgG1) was fused with an anti-folate-binding protein hybrid (IgG2a) to construct bimAbs to target NK cells against NK-resistant ovarian carcinomas. The hybid IgG1/IgG2a bimAb triggered the specific lysis of relevant target cells by resting NK cells, but it was ineffective when CD8+TCR.alpha./.beta.+ cultured cell populations were used as effectors. Only marginal increases of cytolytic activity could be induced by the bimAb when IL-2-activated PBL (i.e., LAK cells) were used as effectors due to high cytolytic activity of these cells against the relevant tumors in the absence of bimAb. The possible use of anti-CD16 or anti-CD3 bimAbs for the development of different cellular immunotherapy strategies against cancer is discussed.

L22 ANSWER 9 OF 39 CA COPYRIGHT 1996 ACS DUPLICATE 9
AN 120:28972 CA
TI Bispecific antibody as a potentiator of tumor cell killing by IL-2-activated lymphocytes
SO Gann Monogr. Cancer Res. (1993), 40(Regulation of Human Cancer by Cytokines), 95-106
CODEN: GMCRDC
AU Yagita, Hideo; Ikeda, Masahiro; Nitta, Taizo; Sato, Kiyoshi; Okumura, Ko; Ishii, Shozo
PY 1993
AB Lymphokine-activated killer (LAK) cells, which can be readily induced from peripheral blood lymphocytes (PBL) by culturing with interleukin 2 (IL-2) and exhibit broad cytotoxicity against various

tumor target cells, have been the subject of clin. trials of adoptive immuno-therapy of cancer patients. Such trials throughout the world, however, have resulted in limited success to date. This appears to result largely from the inefficient recognition of various target cells by LAK cells, the mechanisms for which are still unclear. In order to bypass such an obscure step in the LAK cell-mediated cytotoxicity, the authors used **heteroconjugated** antibodies composed of a monoclonal antibody (mAb) to triggering mols. on LAK cells, such as CD3 and **CD16**, chem. cross-linked with a mAb to glioma-assocd. **antigen**. Such **bispecific antibodies** (BsAb) redirected the LAK cell cytotoxicity against glioma cells in a highly specific and effective manner in vitro. The specific targeting therapy using LAK cells in conjunction with BsAb resulted in a striking clin. efficacy in post-operational treatment of glioma patients as compared with the classical LAK therapy.

L22 ANSWER 10 OF 39 CA COPYRIGHT 1996 ACS DUPLICATE 10
AN 117:129855 CA
TI Bispecific antibodies to a tumor cell and to a cytotoxic cell, method of production, and uses thereof
SO PCT Int. Appl., 61 pp.
CODEN: PIXXD2
IN Ring, David; Shi, Tian Xiang
AI WO 91-US6949 910924
PI WO 9208802 A1 920529
PY 1992
AB A **bispecific antibody** is made by a hybrid hybridoma and is capable of binding to a multiple drug-resistant tumor cell and to a cytotoxic cell. Cells of hybridoma 3G8 secreting a mouse monoclonal antibody to human **Fc**.gamma. receptor III, i.e. **Fc**.gamma.RIII or IgG receptors on cytotoxic cells, were stained with rhodamine 123 and then fused at 1:20 with hybridoma 15D3 cells secreting monoclonal antibodies to multi-drug resistance **antigen**, P-glycoprotein. **Bispecific antibodies** 34.5 and 41.6 mediated lysis of the drug-resistant K562/R7 cells at .apprx.2 times background.

L22 ANSWER 11 OF 39 CA COPYRIGHT 1996 ACS DUPLICATE 11
AN 117:6007 CA
TI Targeted immunostimulation with bispecific reagents
SO PCT Int. Appl., 21 pp.
CODEN: PIXXD2
IN Romet-Lemonne, Jean Loup; Fanger, Michael W.
AI WO 91-US7283 911004
PI WO 9205793 A1 920416
PY 1992
AB Immune response against an antigen is stimulated by administering the antigen in conjunction with a binding agent (e.g. a heteroantibody) specific for an antigen-presenting cell, e.g. a macrophage. The binding agent specifically binds a receptor of the antigen-presenting cell, such as an Fc receptor, without being blocked by the endogenous ligand for the receptor. A bispecific

heteroantibody was prepd. from a monoclonal antibody against human erythrocytes (mono-D, a human anti-RhD antibody) and anti-Fc.gamma.RI antibody 32 (Fc.gamma.RI is the high affinity Fc receptor). The heteroantibody was incubated with erythrocytes, and the heteroantibody-coated erythrocytes were then incubated with adherent monocytes (macrophages). The heteroantibody triggered internalization of the antigen by the macrophages. Enhanced tetanus toxoid presentation by directing tetanus toxoid to human Fc.gamma.R is also described.

L22 ANSWER 12 OF 39 CA COPYRIGHT 1996 ACS DUPLICATE 12
AN 118:57768 CA
TI In vitro cytotoxic targeting by human mononuclear cells and bispecific antibody 2B1, recognizing c-erbB-2 protooncogene product and Fc.gamma. receptor III
SO Cancer Res. (1992), 52(24), 6832-9
CODEN: CNREA8; ISSN: 0008-5472
AU Hsieh-Ma, Sylvia T.; Eaton, Audrey M.; Shi, Tim; Ring, David B.
PY 1992
AB Bispecific murine monoclonal antibody 2B1, possessing dual specificity for the human c-erbB-2 protooncogene product and human Fc.gamma. receptor III (CD16) was evaluated for the ability to promote specific lysis of c-erbB-2-pos. tumor cells in vitro. In short-term 51Cr release assays with human mononuclear cells as effectors and SK-Br-3 human breast cancer cells as targets, neither parental antibody of 2B1 mediated specific lysis, but bispecific antibody was as active as a chem. heteroconjugate, with 5 ng/mL of 2B1 causing half-maximal lysis at an effector/target ratio of 20:1 and 2 ng/mL 2B1 causing half-maximal lysis at an E/T ratio of 40:1. The cytotoxic targeting activity of 2B1 F(ab')2 fragment was the same as that of whole bispecific antibody, and the activity of whole 2B1 was not reduced when assays were performed in 100% autologous human serum, indicating that 2B1 binds effector cells through the CD16-binding site derived from parental antibody 3G8 rather than through its Fc portion. Variable inhibition of 2B1-mediated lysis was obsd. when autologous polymorphonuclear leukocytes from different donors were added to mononuclear effector cells at a 2:1 ratio; this inhibition was overcome at higher antibody concn. 2B1 bispecific monoclonal antibody also mediated targeted cytolysis using whole human blood as a source of effector cells or using effector or target cells derived from ovarian cancer patients.

L22 ANSWER 13 OF 39 CA COPYRIGHT 1996 ACS DUPLICATE 13
AN 117:249723 CA
TI Antitumor effects of a **bispecific antibody** targeting CA19-9 **antigen** and **CD16**
SO Cancer Res. (1992), 52(20), 5713-19
CODEN: CNREA8; ISSN: 0008-5472
AU Garcia de Palazzo, Irma; Holmes, Michele; Gercel-Taylor, Cicek; Weiner, Louis M.
PY 1992
AB Bispecific murine monoclonal antibodies that target tumor and **Fc**.gamma.RIII (**CD16**) can promote relevant tumor

lysis by large granular lymphocytes. For these antibodies to be clin. useful, their properties should be maintained in vivo, where competing human Ig, shed target **antigen**, and shed **CD16** may be encountered. At a min., **bispecific antibody** antitumor effects should be preserved in whole blood. Furthermore, potentiation of tumor lysis should be reflected by demonstrating the ability of **bispecific antibody**-retargeted effector cells to infiltrate and mediate lysis of organized tumor. If these characteristics are demonstrated, and there is evidence of in vivo efficacy of **bispecific antibody**-based therapy in a relevant animal model, further clin. development of such antibodies would be warranted. In this report the ability of CL158 **bispecific antibody** supernatants to mediate lysis of SW948 tumor growing in monolayer is shown to be preserved in the presence of interleukin 2-activated whole blood. When SW948 cells were grown in vitro as multicellular human tumor spheroids, incubation with interleukin 2-activated lymphocytes (LAK cells) and CL158 led to structural and widespread necrosis. This was dependent on CL158 and resistant to competition by pooled human Ig or interleukin 2-exposed whole blood. These effects were not promoted by the monospecific antibodies produced by the parent clones of CL158 and were not obsd. when the IgG2a variant of CA19-9 antibody, which mediates conventional antibody-dependent cellular cytotoxicity, was used instead of its bispecific deriv. To examine the efficacy of **bispecific antibody**-based treatments on in vivo tumor, scid mice bearing early s.c. SW948 xenografts were treated with interleukin 2 for 5 consecutive days, supplemented by 3 i.v. injections of 107 human LAK cells and various antibodies. Treatment of mice bearing SW948 tumors with LAK cells did not retard tumor growth, but when CL158 was added, delays in tumor growth were obsd. Tumor growth delay required treatment with both LAK cells and the **bispecific antibody**. Treatment with the IgG2a variant of CA19-9 antibody, alone or with LAK cells, had no effects on tumor growth. It is clear that human LAK cell treatment of animals bearing early, established s.c. tumors is enhanced by the addn. of **bispecific antibodies** with relevant binding characteristics. When compared with the IgG2a isotype variant of CA19-9 monoclonal antibody, this **bispecific antibody** offers the advantages of preservation of activity in physiol. conditions, infiltration and disruption of organized tumor in vitro, and antitumor effects in a relevant xenograft model.

L22 ANSWER 14 OF 39 CA COPYRIGHT 1996 ACS DUPLICATE 14
AN 118:99977 CA
TI Redirected targeting of LDL to human monocyte Fc.gamma. receptors with bispecific antibodies
SO Arterioscler. Thromb. (1992), 12(10), 1131-8
CODEN: ARTTE5; ISSN: 1049-8834
AU Morganelli, Peter M.; Kitzmiller, Tamar J.; Hemmer, Ruth; Fanger, Michael W.
PY 1992
AB Recent studies indicate that LDL-immune complexes consisting of

anti-LDL antibodies bound to LDL may contribute to macrophage foam cell development by uptake through IgG **Fc** receptors. As human mononuclear phagocytes possess 3 structurally and functionally distinct classes of IgG **Fc** receptors, a system was developed whereby the effects of LDL-immune complexes could be studied with respect to each type of IgG **Fc** receptor. Novel **bispecific antibodies** consisting of anti-**Fc**.gamma. receptor antibodies linked to anti-LDL antibodies were used to prep. bispecific LDL-immune complexes for targeting to specific **Fc**.gamma. receptors. In this report, the effects of bispecific LDL-immune complexes directed to **Fc**.gamma. receptor types I, II, and III were studied primarily with monocytes and were compared with the effects of similarly prepd. bispecific complexes that targeted LDL to human leukocyte **antigen** (HLA) class I **antigens**. Each type of **bispecific antibody** was effective in targeting 125I-labeled LDL to its resp. site on the cell surface. Using fluorophore-labeled LDL and flow cytometry, bispecific complexes directed to **Fc**.gamma. receptor types I or II but not to HLA class I **antigens** caused a 2-7-fold increase in cell-assocd. fluorescence relative to control cells treated with LDL in the absence of **bispecific antibody**. Uptake occurred in the presence of excess unlabeled LDL, acetylated LDL, and antioxidants. That the bispecific complexes triggered metabolic uptake was supported by studies of kinetics and temp. dependence. By the use of 125I-labeled complexes, metabolic degrdn. of LDL was demonstrated in assocn. with each of the 3 types of **Fc**.gamma. receptors. Thus, bispecific LDL-immune complexes directed to **Fc** .gamma. receptors were taken up by monocytes, whereas similar bispecific complexes directed to HLA class I **antigens** were not. Native LDL receptors and scavenger receptors were not involved. Uptake through each of the different **Fc**.gamma. receptors was assocd. with metabolic degrdn. of LDL. Bispecific reagents should be extremely useful for the anal. of pathways of lipoprotein metab. assocd. with each type of **Fc**.gamma. receptor and(or) other cellular structures.

L22 ANSWER 15 OF 39 CA COPYRIGHT 1996 ACS DUPLICATE 15
AN 117:88257 CA
TI Bispecific monoclonal antibody regulation of Fc.gamma.RIII-directed tumor cytotoxicity by large granular lymphocytes
SO Cell. Immunol. (1992), 142(2), 338-47
CODEN: CLIMB8; ISSN: 0008-8749
AU Garcia de Palazzo, Irma; Kitson, Joanne; Gercel-Taylor, Cicek; Adams, Stacy; Weiner, Louis M.
PY 1992
AB Bispecific monoclonal antibodies (BsMAbs) prepd. by somatic cell fusion bind monovalently to their targets and yet are extremely potent enhancers of target cell lysis by relevant effector cells. The mechanisms underlying this efficiency are not known. To investigate this property, the ability was studied of selected antibodies to modulate potentiation of tumor lysis by a **bispecific antibody** (CL158) which targets

Fc.gamma.RIII-expressing cells, via the 3G8 epitope, to malignant cells expressing CA19-9 **antigen**. Antibodies directed against the 3G8 and B73.1 epitopes of **Fc** .gamma.RIII efficiently inhibited BsMAb-mediated SW948 tumor cell lysis by interleukin-2 (IL-2)-activated lymphocytes (PBLs). Unexpectedly, Leu 19 antibody reversed antibody-dependent but not antibody-independent lysis of 51Cr-labeled SW948 cells by IL-2-activated PBLs in a concn.-dependent fashion. Leu 19 binds to CD56, a neural cell adhesion mol. (N-CAM) isoform expressed by large granular lymphocytes (LGLs). The effects of Leu 19 on **bispecific antibody** promotion of lysis were due to competition for binding to the 3G8 epitope of **Fc** .gamma.RIII and led to inhibition of binding between LGLs and SW948 cells. Leu 19 did not inhibit antibody-dependent lysis by the monospecific, bivalent IgG2a variant of CA19-9 antibody. Thus, competition assays can be useful in dissecting the relevant mechanisms underlying BsMAb-promoted lysis. Steric constraints between effector cell trigger mols. (i.e., **Fc**.gamma.RIII) and CAM such as N-CAM may regulate the function of these mols.

L22 ANSWER 16 OF 39 CA COPYRIGHT 1996 ACS DUPLICATE 16
AN 118:5417 CA
TI Bispecific anti-human red blood rhesus-D antigen .times. anti Fc.gamma.RI targeted antibody-dependent cell-mediated cytotoxicity and phagocytosis by mononuclear leukocytes
SO Clin. Exp. Immunol. (1992), 89(2), 310-14
CODEN: CEXIAL; ISSN: 0009-9104
AU Deramoudt, F. X.; Gilard, C.; Lepine, N.; Alonso, J. M.; Romet-Lemonne, J. L.
PY 1992
AB The **Fc** receptor mediated antibody-dependent cell-mediated cytotoxicity (ADCC) and phagocytosis induced by **bispecific antibody** (BsAb) to the high-affinity **Fc** receptor for IgG (**Fc**.gamma.RI) and to human red blood group **antigen** RhD were studied in vitro, using human mononuclear leukocytes as effector cells. The results were compared with those obtained by using a human monoclonal IgG1 anti-RhD used alone and a ref. human polyclonal anti-RhD antibody. The effect of non-specific human IgG on **Fc**R-mediated functions by mononuclear leukocytes was checked. The results demonstrate that BsAb presents a high resistance of **Fc**-mediated function to blockade by non-specific human IgG compared with that of both polyclonal and monoclonal anti-RhD antibodies. These results further encourage possible clin. application of **bispecific antibody** in passive immunotherapy.

L22 ANSWER 17 OF 39 CA COPYRIGHT 1996 ACS DUPLICATE 17
AN 117:109822 CA
TI Adoptive immunotherapy with bispecific antibodies: targeting through macrophages
SO Res. Immunol. (1992), 143(1), 95-9
CODEN: RIMME5; ISSN: 0923-2494
AU Chokri, M.; Girard, A.; Borrelly, M. C.; Oleron, C.; Romet-Lemonne,

J. L.; Bartholeyns, J.
PY 1992
AB The authors report on 2 applications of **bispecific antibodies** to enhance the antitumoral function of human macrophages: (1) use of rhuIFN.gamma. (recombinant human interferon .gamma.) encapsulated in human red blood cells coated with anti-**Fc**.gamma.RI (**Fc** receptor for IgG)/anti-blood group substance RhD+ **bispecific antibodies** to target and to activate human macrophages; encapsulated rhuIFN.gamma. was more potent than free IFN.gamma. in activating mature macrophages in vitro, demonstrating the efficacy of this delivery system to initiate in situ activation of macrophages and also to maintain a high antitumoral efficacy of macrophages with less side effects than after systemic injection of IFN.gamma.; (2) targeting of activated macrophages to tumors by **bispecific antibodies** directed against macrophage **Fc**.gamma.RI and against human adenocarcinoma **antigen**; differentiated human macrophages became cytotoxic for human adenocarcinoma in vitro and in vivo (tumors implanted in nude mice) when activated by rhuIFN.gamma.; this effect was increased in the presence of **bispecific antibodies**. These 2 approaches were aimed at increasing the efficacy of cellular immunotherapies using activated macrophages as effector cells (macrophage-activated killer, or MAK). **Bispecific antibodies** could increase specific homing and activation of cytotoxic MAK effectors at tumor sites.

L22 ANSWER 18 OF 39 CA COPYRIGHT 1996 ACS DUPLICATE 18
AN 115:90564 CA
TI Bispecific heteroantibodies with dual effector functions
SO PCT Int. Appl., 26 pp.
CODEN: PIXXD2
IN Fanger, Michael W.; Guyre, Paul M.; Ball, Edward D.
AI WO 90-US5981 901018
PI WO 9105871 A1 910502
PY 1991
AB **Bispecific antibodies** which react both with the high-affinity **Fc**.gamma. receptor (CD64 **antigen**) of human effector cells and with a target cell surface **antigen** are disclosed. Binding of the mols. to the **Fc** receptors found on effector cells is not blocked by human IgG. The mols. are useful for targeting human effector cells (e.g. macrophages) against cells bearing this target **antigen**. For this purpose, bispecific mols. can be constructed contg. the binding region derived from an anti-**Fc**.gamma. receptor antibody and the binding region derived from an antibody specific for the target **antigen**. Targeted effector cells can be used to destroy cells bearing the target cell surface **antigen** by cell-mediated antibody-dependent cytolysis and by complement-fixation. Thus, Fab from a monoclonal antibody to the human monocyte high-affinity receptor was conjugated to monoclonal IgM to the CD15 cell-surface **antigen**. Monocytes, HL-60 leukemia cells, and the heteroantibody were incubated together for 18 h at 37.degree.. Monocytes alone caused 5-20% killing, monocytes

plus heteroantibody caused 20-50% killing, and monocytes plus heteroantibody plus human serum caused 50-80% killing.

L22 ANSWER 19 OF 39 CA COPYRIGHT 1996 ACS DUPLICATE 19
AN 115:64752 CA
TI Anti-Rh(D) heteroantibodies and pharmaceutical composition containing same for drug targeting and therapy using macrophages
SO PCT Int. Appl., 22 pp.
CODEN: PIXXD2
IN Fanger, Michael; Lazard, Florence; Romet-Lemonne, Jean Loup
AI WO 90-FR757 901019
PI WO 9105800 A1 910502
PY 1991
AB Chimeric antibodies comprise all or part of anti-Rh(D) blood-group substance antibody linked with all or part of an antibody to a receptor for Fc fragment of Igs that is not blocked by IgG. These chimeric antibodies are bound to erythrocytes encapsulating, e.g. macrophage activators, antiinfective agents, and anticancer agents, via the Rh(D) surface antigen on the erythrocytes, and the complexes target macrophages and are thus useful in therapies involving macrophages. The F(ab')2 fragment of monoclonal antibody H2D5D2 (anti D) was coupled to the FAb' fragment of monoclonal antibody 32.2 (anti Fc.gamma.RI). This chimeric antibody was reacted with Rh-pos. erythrocytes loaded with .gamma. interferon. U937 tumor cells were inhibited using human macrophages and the complex.

L22 ANSWER 20 OF 39 CA COPYRIGHT 1996 ACS DUPLICATE 20
AN 115:277836 CA
TI Fc receptors for IgG (Fc.gamma.Rs) on human monocytes and macrophages are not infectivity receptors for human immunodeficiency virus type 1 (HIV-1): studies using bispecific antibodies to target HIV-1 to various myeloid cell surface molecules, including the Fc.gamma.R
SO Proc. Natl. Acad. Sci. U. S. A. (1991), 88(21), 9593-7
CODEN: PNASA6; ISSN: 0027-8424
AU Connor, R. I.; Dinces, N. B.; Howell, A. L.; Romet-Lemonne, J. L.; Pasquali, J. L.; Fanger, M. W.
PY 1991
AB **Fc**.gamma.Rs (**Fc**.gamma.RI, **Fc** .gamma.RII, and **Fc**.gamma.RIII) are highly expressed on human mononuclear phagocytes and function in the clearance of immune complexes and opsonized pathogens. The authors examd. the role of **Fc**.gamma.R in mediating antibody-dependent clearance of HIV-1 by human monocytes and monocyte-derived macrophages by using **bispecific antibodies** (BsAbs) to independently target the virus to **Fc**.gamma.RI, **Fc**.gamma.RII, or **Fc**.gamma.RIII. Virus prodn. was markedly reduced in monocytes cultured with strain HIV-1IIIB opsonized with BsAbs that target the virus to either **Fc**.gamma.RI or **Fc** .gamma.RII compared to monocytes cultured with virus in the absence of BsAbs or in the presence of BsAbs that target the virus to non-**Fc**.gamma.R surface **antigens** (CD33 and HLA-A,B,C). These results were confirmed using the monotropic isolate HIV-1JRFL.

Interaction of HIV-1JRFL with **Fc**.gamma.RII on human monocytes and **Fc**.gamma.RI, **Fc**.gamma.RII, or **Fc**.gamma.RIII on monocyte-derived macrophages resulted in markedly reduced levels of virus prodn. in these cultures. Moreover, HIV-1 infection of monocytes and monocyte-derived macrophages was completely blocked by anti-CD4 monoclonal antibodies, indicating that interaction with CD4 is required for infectivity even under conditions of antibody-mediated binding of HIV-1 to **Fc**.gamma.R. Thus, it is proposed that highly opsonized HIV-1 initiates high-affinity multivalent interactions with **Fc**.gamma.R that trigger endocytosis and intracellular degrdn. of the antibody-virus complex. At lower levels of antibody opsonization, there are too few interactions with **Fc** .gamma.R to initiate endocytosis and intracellular degrdn. of the antibody-virus complex, but there are enough interactions to stabilize the virus at the cell surface, allowing antibody-dependent enhancement of HIV-1 infection through high-affinity CD4 interactions. However, interaction of highly opsonized HIV-1 with **Fc**.gamma.Rs through BsAbs may reduce viral infectivity through **Fc**.gamma.R-mediated cytotoxic mechanisms and, therefore, BsAbs offer promise as therapeutic reagents in HIV-1 infections.

L22 ANSWER 21 OF 39 CA COPYRIGHT 1996 ACS DUPLICATE 21
AN 115:133642 CA
TI Functions of the various human IgG Fc receptors in mediating killing of Toxoplasma gondii
SO J. Immunol. (1991), 146(9), 3145-51
CODEN: JOIMA3; ISSN: 0022-1767
AU Erbe, David V.; Pfefferkorn, E. R.; Fanger, Michael W.
PY 1991
AB The three types of IgG **FcR** (**Fc**.gamma.RI, **Fc**.gamma.RII, **Fc**.gamma.RIII) on human leukocytes play an important role in elimination of antibody-coated infectious agents. To further understand the role of the different **Fc** .gamma.R in mediating this killing, the authors examd. the ability of human myeloid and lymphoid cells to kill the protozoan T. gondii in the presence of anti-toxoplasma IgG or **bispecific antibodies**. Although human myeloid cells (monocytes, macrophages, neutrophils, and eosinophils) all lysed unsensitized T. gondii, killing by these cells was significantly enhanced by opsonization with antitoxoplasma rabbit IgG. Human lymphocytes, however, did not lyse T. gondii unless the parasites were coated with antibody. The role of antibody and **Fc**.gamma.R in mediating ADCC of T. gondii was then examd. using **bispecific antibodies** made by chem. crosslinking Fab fragments of antitoxoplasma antibodies to Fab fragments of antibodies specific for human leukocyte surface **antigens** (Ag), including **Fc**.gamma.R. Thus, simultaneous binding of these bispecifics to parasites and effector cells allowed an evaluation of killing when T. gondii were targeted to each Ag independently. Bispecifics which targeted T. gondii to **Fc**.gamma.RI, II or III enhanced lysis by monocytes. However, similar results were obtained

with bispecifics targeting T. gondii to non-**Fc**.gamma.R Ag (CD11b or .beta.2-microglobulin) on monocytes. Likewise, polymorphonuclear leukocytes mediated significantly more lysis in the presence of bispecifics linking T. gondii to **Fc** .gamma.RII, **Fc**.gamma.RIII, or the two non-**Fc** .gamma.R Ag CD11b and .beta.2-microglobuln. Thus, although human myeloid cells did not require antibody-**Fc**.gamma.R triggering to kill T. gondii, antibody appeared to enhance lysis by capturing and directing the parasites to the effector cell surface. Human lymphocytes, in contrast, mediated significant lysis of T. gondii only in the presence of bispecifics targeting T. gondii to **Fc**.gamma.RIII, indicating a requirement for specific triggering of **Fc**.gamma.RIII for killing by large granular lymphocytes. Consequently, using bispecifics to compare targeting to specific Ag, both non-**Fc**.gamma.R and **Fc** .gamma.R, allowed detn. of the role of antibody-**Fc**.gamma.R interactions in T. gondii killing.

L22 ANSWER 22 OF 39 CA COPYRIGHT 1996 ACS DUPLICATE 22
AN 116:39331 CA
TI Target cell-induced T cell activation with bi- and trispecific antibody fragments
SO Eur. J. Immunol. (1991), 21(10), 2431-5
CODEN: EJIMAF; ISSN: 0014-2980
AU Jung, Gundram; Freimann, Uwe; Von Marschall, Zofia; Reisfeld, Ralph A.; Wilmanns, Wolfgang
PY 1991
AB Previously, the authors proposed a concept for tumor immunotherapy in which two different **bispecific antibody** conjugates, an anti-target .times. anti-CD3 and an anti-target .times. anti-CD28 conjugate, induce the activation of resting human T cells upon binding to the resp. tumor target cells. After in vivo application of these reagents, this model of a target cell-induced T activation envisages the destruction of target cells by in situ activated T cells. Obviously however, for in vivo application, the use of **Fc**-free antibody fragments is mandatory to prevent binding of the conjugates to **Fc** receptor-pos. cells which would lead to **Fc**-mediated T cell activation. Here is reported a simplification of published procedures for the generation of bispecific Fab-hybrid fragments, univalent for each **antigen**. It is demonstrated that an anti-target .times. anti-CD3/anti-target .times. anti-CD28 combination of such hybrids, as well as an identical combination of covalently coupled F(ab')2 fragments, mediate target cell-induced T cell activation in an in vitro test system. Thus, these reagents may be capable of inducing an in situ activation of human T cells upon systemic in vivo application according to the concept outlined above. A trispecific conjugate with anti-target, anti-CD3- and anti-CD28 specificity appears to be unsuitable for this purpose because it activates resting T cells in sol. form without requiring immobilization through binding via its anti-target portion.

L22 ANSWER 23 OF 39 CA COPYRIGHT 1996 ACS DUPLICATE 23

AN 116:126516 CA
TI Enhancement of cytolytic activity in lymphokine-activated killer (LAK) cells using bispecific F(ab')2
SO Nippon Ika Daigaku Zasshi (1991), 58(6), 663-72
CODEN: NIDZAJ; ISSN: 0048-0444
AU Azuma, Arata
PY 1991
AB F(ab')2 bispecific antibodies which consisted of NCC-LU246 immunized by a small cell lung carcinoma (SCLC) cell line of Lu24 and either OKT3 specific for CD3 or 3G8 specific for Fc .gamma. receptor III potentiated the cytolytic activity of peripheral blood mononuclear cells (PBMC) or LAK cells from healthy volunteers against H69, a SCLC line. Cytolytic activity against H69 of natural killer (NK) cells was largely potentiated by interleukin-2 treatment (NK-LAK). T cells in PBMC showed little cytolytic activity against H69, and T-LAK exhibited significantly less activity than NK-LAK. Bispecific antibody with 3G8 did not elevate the cytolytic activity of NK-LAK, and that with OKT3 elevated the activity of T-LAK. The OKT3 bispecific antibody also elevated the cytolytic activity of LAK cells derived from whole PBMC. A higher ratio of cytolytic activity potentiation was obsd. with OKT-3 bispecific antibody than 3G8 bispecific antibody in the Winn assay. The bispecific antibodies were effective with cytolytic cells derived from tumor-bearing patients, but the activity was lower than in healthy volunteers.

L22 ANSWER 24 OF 39 CA COPYRIGHT 1996 ACS DUPLICATE 24
AN 116:171871 CA
TI Retrovirus-derived shuttle vectors in the generation of murine bispecific MoAbs to be used in ex vivo and in vivo immunotherapy
SO Ann. Ist. Super. Sanita (1991), 27(1), 133-7
CODEN: AISSAW; ISSN: 0021-2571
AU Demonte, L. B.; Dellabona, P.; Nistico, P.; Tecce, R.; Camagna, M.; Reyna, A.; Momo, M.; Natali, P. G.; Mariani, M.; Malavasi, F.
PY 1991
AB Mouse monoclonal antibodies specific for CD3, **FcR**, and a melanoma assocd. **antigen** were produced. Drug resistance of such hybridomas was obtained tranfecting them with plasmids contg. genes conferring specific resistance. Retrovirus-derived shuttle vectors with high transfection efficiency were used for transfection. Hybridomas were than fused and **bispecific antibody** producing cells selected. Purifn. was performed by HPLC. Such **bispecific antibodies** can be used in ex vivo and in vivo immunotherapy.

L22 ANSWER 25 OF 39 CA COPYRIGHT 1996 ACS DUPLICATE 25
AN 115:6600 CA
TI Interleukin-2 activated T cells (T-LAK) express **CD16 antigen** and are triggered to target cell lysis by **bispecific antibody**
SO Immunol. Lett. (1991), 28(1), 31-7
CODEN: IMLED6; ISSN: 0165-2478
AU Nitta, Taizo; Nakata, Motomi; Yagita, Hideo; Okumura, Ko
PY 1991

AB Human mononuclear cells from healthy donors were cultured with 100 U/mL recombinant interleukin-2 (rIL-2) for up to 5 wk and tested at short and long activation times for the ability to mediate CD3 and CD16 targeted cytotoxicity using chem. cross-linked bispecific antibodies. At each period, lymphokine-activated killer activity was augmented with the use of bispecific antibodies (BA), whereas interestingly enough, at later periods (4-5 wk) when CD16 pos. lymphocytes are not present, as detd. by flow cytometry, CD16 targeted cytotoxicity was induced. The authors suspected the possibility of CD16 expression on activated T cells and have purified the T cell subpopulations to see the targeted cytotoxicity. Populations enriched for T cells by Percoll d. centrifugation, treatment with anti-CD16 plus complement or sorting for CD5+ cells, were all able to mediate CD16 targeted cytotoxicity following activation with rIL-2. These data suggest that IL-2 activated T cells express CD16 in addn. to CD3.

L22 ANSWER 26 OF 39 CA COPYRIGHT 1996 ACS DUPLICATE 26
AN 113:209907 CA
TI Manufacture of antibody specific to two different antigens
SO Jpn. Kokai Tokkyo Koho, 8 pp.
CODEN: JKXXAF
IN Azuma, Takachika; Nitta, Taizo; Okumura, Yasushi
AI JP 88-227190 880910
PI JP 02076899 A2 900316 Heisei
PY 1990
AB A method for prepg. a bispecific antibody involves: (a) treating 2 F(ab')2 segments prepd. from antibodies recognizing different antigens with a dithiol reducing agent [i.e. dithiothreitol or 5,5'-dithiobis(2-nitrobenzoic acid)] in the presence of chelators (EDTA) to form Fab' segments, (b) activating the Fab' segments with excess thiol group activators, and (c) reacting the Fab' segments from b and those from a to form a F(ab')2 segment recognizing 2 different antigens. Prepn. of a bispecific antibody [F(ab')2] recognizing 4-hydroxy-3-nitrophenylacetic acid (Ac group) as well as 2,4-dinitrophenol is given as an example.

L22 ANSWER 27 OF 39 CA COPYRIGHT 1996 ACS DUPLICATE 27
AN 114:4574 CA
TI Potentiation of tumor lysis by a **bispecific antibody** that binds to CA19-9 **antigen** and the **Fc**.gamma. receptor expressed by human large granular lymphocytes
SO Cancer Res. (1990), 50(22), 7123-8
CODEN: CNREA8; ISSN: 0008-5472
AU Garcia de Palazzo, Irma; Gercel-Taylor, Cicek; Kitson, Joanne; Weiner, Louis M.
PY 1990
AB A bispecific, monovalent monoclonal antibody was prepd., purified, and evaluated for its in vitro effects. The IgG1-secreting hybridoma line 3G8 (.alpha.-human Fc.gamma.R III) was fused with the hybridoma line CA19-9, which produces an IgG1 antibody that binds to a glycoprotein shed by gastrointestinal cancers. Multiple clones

with bispecific binding properties were identified. CA19-9 .times. 3G8 clonal supernatants and purified antibody, but not the parent antibodies, efficiently mediated specific in vitro lysis of cells of the SW948 line by human large granular lymphocytes (LGLs). Human serum-resistant target cell lysis augmentation at low effector:target ratios was seen using picogram amts. of antibody. In contrast, the igG2.alpha. variant of CA19-9, which also promotes ADCC by LGLs, did not augment lysis of SW948 cells when effectors were preincubated with human serum. This bispecific, monovalent monoclonal antibody is an efficient promoter of the anti-tumor effects of LGLs in physiol. concns. of human serum.

L22 ANSWER 28 OF 39 CA COPYRIGHT 1996 ACS DUPLICATE 28
AN 113:57073 CA
TI Enhanced antigen immunogenicity induced by bispecific antibodies
SO J. Exp. Med. (1990), 171(6), 1957-63
CODEN: JEMEAV; ISSN: 0022-1007
AU Snider, Denis P.; Kaubisch, Andreas; Segal, David M.
PY 1990
AB The binding of protein **antigens** to APC with heterocrosslinked **bispecific antibodies** (HBAs) enhances their processing and presentation to Th cells in vitro. Mice immunized with hen egg lysozyme (HEL) in the presence or absence of HBA, were followed for antibody prodn. after the primary challenge and after a secondary boost. HBAs that bind **antigen** to MHC class I or II mols., to **Fc**.gamma.R, but not to surface IgD, enhance the immunogenicity of HEL. HBAs that bound HEL to MHC class II mols., for example, decreased the amt. of **antigen** required to elicit a primary anti-HEL antibody response in mice by 300-fold, and the amt. required to prime for a secondary response by 103-104-fold. In fact, HBAs were as effective as incomplete Freund"s adjuvant in generating antibody responses. Since adjuvants cannot be used in humans, HBAs could prove useful for immunizing people, esp. in cases where, due to scarcity or toxicity, minute doses of **antigen** must be used.

L22 ANSWER 29 OF 39 CA COPYRIGHT 1996 ACS DUPLICATE 29
AN 115:181001 CA
TI Variable linking region immunogenicity using malarial peptide carrier protein conjugates of defined composition
SO Immunol. Lett. (1990), 26(3), 285-90
CODEN: IMLED6; ISSN: 0165-2478
AU Jones, Graham Lloyd; Spencer, Lisa; Mollard, Rodney; Saul, Allan
PY 1990
AB Thirty-five overlapping petides from both conserved and variable parts of the N-terminal region of a malarial merozoite surface **antigen** (MSA2) were synthesized using solid phase chem. All peptides were synthesized with an added N-terminal cysteine and purified by reverse-phase HPLC to facilitate coupling to a carrier protein diphtheria toxoid (DT) using the **hetero-bifunctional** reagent maleimidocaproloxysuccinimide (MCS). Mice were immunized with these peptide-DT conjugates using Freund's

complete adjuvant (**FCA**). The immune response of these mice was tested against peptide hapten, carrier protein (DT) and the linking region itself, using ELISA. Although there was great variation in the immune response to each part of the immunogen construct, no correlation could be seen between each set of responses.

L22 ANSWER 30 OF 39 CA COPYRIGHT 1996 ACS DUPLICATE 30
AN 110:171368 CA
TI Inhibition of cytotoxic T cell lysis by anti-CD8 monoclonal antibodies: studies with CD3-targeted cytolysis of nominal antigen-negative targets
SO J. Immunol. (1989), 142(7), 2200-6
CODEN: JOIMA3; ISSN: 0022-1767
AU Quinones, Ralph R.; Segal, David M.; Henkart, Pierre; Perez, Pilar; Gress, Ronald E.
PY 1989
AB The function of the CD8 mol. in lympholysis mediated by cytotoxic T cells was investigated by examg. possible contributions of ligands of the target cell to the inhibition of lysis obsd. with CD8-specific mAb. In order to evaluate a variety of target cells, including those not expressing the nominal **antigen** (NA) for which the CTL was specific, lysis was effective by crosslinking the CTL and the target cells with anti-CD3 mAb. Such CD3 redirected cytotoxicity was inhibited by anti-CD8 mAb when low anti-CD3 mAb concns. were used. The possibility that inhibition by anti-CD8 mAb resulted in competition for the **Fc** receptor between the anti-CD3 mAb and anti-CD8 mAb was eliminated by targeting TNP-modified cells with an antibody **heteroconjugate** prepd. from Fab fragments of anti-CD3 and anti-DNP antibodies. Inhibition of the lysis of target cells not expressing NA including those deficient in class I expression, demonstrated that neither NA nor class I expression was required for anti-CD8 mAb inhibition. Whether the anti-CD8 mAb inhibition required CD8 **antigen** interaction with any ligand on the target cell was further investigated by measuring exocytosis of enzyme granule from CTL activated with CD3-coated polystyrene beads. CD8-specific mAb inhibited such CTL activation in this target cellfree system. A CD8(+), MHC class II-specific CTL clone, was used to show differential inhibition by anti-CD8 mAb, depending on the target cell, therefore providing evidence that anti-CD8 mAb binding does not generate an abs. off signal. Thus, anti-CD8 mAb affect the lytic process independent of the recognition of a ligand on the target cell by CD8.

L22 ANSWER 31 OF 39 CA COPYRIGHT 1996 ACS DUPLICATE 31
AN 111:76057 CA
TI Efficiency of antigen presentation after antigen targeting to surface IgD, IgM, MHC, Fc.gamma.RII, and B220 molecules on murine splenic B cells
SO J. Immunol. (1989), 143(1), 59-65
CODEN: JOIMA3; ISSN: 0022-1767
AU Snider, Denis P.; Segal, David M.

PY 1989
AB Bispecific **heteroconjugate** antibodies can bind sol. protein **antigens** to **antigen**-presenting cells and thereby enhance **antigen** presentation. Such antibodies were used to bind hen egg lysozyme (HEL) to various structures on the surface of normal splenic B cells to det. which structures would provide the best targets for enhanced presentation. HEL was presented efficiently to hybridoma T cells if bound to sIgD, sIgM, or class I or II MHC mols., but not at all if bound to **Fc** .gamma.RII, or B220 mols. on B cells. The efficiency of presentation of HEL was measured as a function of the amt. of 125I-HEL bound per cell. HEL was presented with 5-10-fold greater efficiency when bound to sIg, than when bound to MHC mols. When compared on the basis of the amt. of HEL bound, sIgD and sIgM functioned equally as target structures, as did class I and class II MHC mols. Large amts. of HEL bound to B220, but no presentation resulted, indicating that focusing HEL to the **antigen** -presenting cell surface was not sufficient for presentation to occur. HEL was internalized rapidly and in large amts. when bound to sIgD or sIgM, but slowly and in small amts., when bound to class I or class II MHC mols. Thus, a rapid rate of internalization may in part explain the high efficiency of **antigen** presentation after binding to sIg. However, the small amt. of HEL internalized via MHC mols. was utilized efficiently for presentation. Thus, sIgM and sIgD serve equally on normal B cells to focus and internalize **antigens** and enhance **antigen** presentation, but class I or class II MHC mols. can also be used to internalize **antigens** and enhance **antigen** presentation, perhaps by a sep. intracellular processing pathway.

L22 ANSWER 32 OF 39 CA COPYRIGHT 1996 ACS DUPLICATE 32
AN 108:202889 CA
TI Lysis of cells infected with HIV-1 by human lymphocytes targeted with monoclonal antibody heteroconjugates
SO J. Immunol. (1988), 140(8), 2609-13
CODEN: JOIMA3; ISSN: 0022-1767
AU Zarling, Joyce M.; Moran, Patricia A.; Grosmaire, Laura S.; McClure, Jan; Shriver, Kathleen; Ledbetter, Jeffrey A.
PY 1988
AB The present study was undertake to det. whether human peripheral blood lymphocytes (PBL) can be specifically focused to lyse cells infected with human immunodeficiency virus-1 (HIV-1) by monoclonal antibodies (mAb) heteroconjugates that can bridge target and effector cells. A mAb directed against the central portion of HIV-1 glycoprotein gp110 was chem. cross-linked to a mAb directed against the CD3/TCR complex or to a mAb directed against the CD16 Fc.gamma.-R expressed on large granular lymphocytes (LGL). HIV-1-infected cells, but not uninfected cells, were lysed to a greater extent by PBL in the presence of the gp110 .times. CD3 or the gp110 .times. CD16 antibody heteroconjugate than in the presence of the single antibodies or a mixt. of the mAb comprising the heteroconjugates. Pretreatment of PBL with anti-CD3 or IL-2

augments their ability to lyse HIV-1-infected cells in the presence of the heteroconjugates. Lysis by anti-CD3-activated PBL in the presence of the gp110 .times. CD3 heteroconjugate was mediated by CD8+-enriched T cells, whereas lysis by IL-2-treated PBL in the presence of the gp110 .times. CD16 heteroconjugate is mediated by PBL enriched for CD16+ cells, which are primarily LGL. PBL from asymptomatic, HIV-1-infected seropos. donors were functional in lysing HIV-1-infected cells in the presence of the antibody heteroconjugates. Such antibody heteroconjugates, which can target T cells or LGL to lyse HIV-1-infected cells, may be of prophylactic or therapeutic value in HIV-1-infected individuals.

L22 ANSWER 33 OF 39 CA COPYRIGHT 1996 ACS DUPLICATE 33
AN 110:93170 CA
TI T cell-mediated immunity in recipients of HSV-2 and HIV-1 vaccines and lysis of HIV-1 infected cells targeted by monoclonal antibody heteroconjugates
SO UCLA Symp. Mol. Cell. Biol., New Ser. (1988), 84(Technol. Adv. Vaccine Dev.), 661-9
CODEN: USMBD6; ISSN: 0735-9543
AU Zarling, Joyce M.; Moran, Patricia A.; Corey, Lawrence; Hu, Shiu Lok; Ledbetter, Jeffrey A.
PY 1988
AB Studies were undertaken to det. (a) whether immunization of humans with a herpes simplex virus-2 (HSV-2) glycoprotein-subunit vaccine results in the priming of HSV-specific T helper (Th) and cytotoxic T cells (CTL), (b) whether immunization of chimpanzees with a recombinant vaccinia virus expressing human immunodeficiency virus-1 (HTV-1) envelope (env) glycoproteins results in the priming of HIV-specific Th and CTL, and (c) whether human T cells or large granular lymphocytes (LGL) can be focused, by monoclonal antibody **heteroconjugates**, to lyse HIV-1 infected cells. PBL from humans immunized with an HSV-2 glycoprotein-subunit vaccine proliferate following stimulation with HSV glycoproteins and HSV-specific CTL are also primed. Chimpanzees immunized with a recombinant-vaccinia virus expressing HIV-1 have HIV-specific Th and also env CTL that lyse cells expressing env. CD8+ and **CD16** + PBL from both HIV-1 seroneg. and seropos. donors can be focused to kill HIV-1 infected cells by monoclonal antibody **heteroconjugates** contg. a neutralizing antibody to HIV-1 cross-linked to antibodies against lymphocyte cell surface **antigens.**

L22 ANSWER 34 OF 39 CA COPYRIGHT 1996 ACS DUPLICATE 34
AN 109:90913 CA
TI Cross linking of anti-B16 melanoma monoclonal antibodies to lymphokine activated killer (LAK) cells: possible role in the therapy of B16 melanoma
SO Clin. Exp. Metastasis (1988), 6(5), 387-400
CODEN: CEXMD2; ISSN: 0262-0898
AU Eisenthal, Avi; Rosenberg, Steven A.
PY 1988
AB Using succinimidyl 4-(N-maleimidomethyl)cyclohexane-1-carboxylate

(SMCC) as a **heterobifunctional** reagent, anti-B16 melanoma monoclonal antibody was cross-linked to lymphokine-activated killer (LAK) cells, independent of the **Fc** receptor. The conditions of such linkage were optimized so that the cytotoxic properties of LAK cells, as measured in a 4 h Cr release assay against fresh tumor cells, were preserved. Covalent crosslinking of anti-B16 antibody to LAK cells preserved the reactivity of this antibody to **antigens** on B16 melanoma cells, and preserved the cytotoxic properties of the antibody-bound LAK cells to lyse B16 tumor cells and other tumor cells in vitro. Crosslinking antibody remained active on the surface of LAK cells for as long as 24 h after the completion of binding. Treatment of established B16 melanoma pulmonary or s.c. tumors with LAK cells cross-linked to anti-B16 melanoma monoclonal antibody did not alter their therapeutic efficacy over untreated cells. The possible explanations of these in vivo observations and suggested approaches to increase the efficacy of the cross-linked LAK cells are discussed.

L22 ANSWER 35 OF 39 CA COPYRIGHT 1996 ACS DUPLICATE 35
AN 106:194200 CA
TI Demonstration of the platelet Fc receptor by using a photosensitive heterobifunctional cross-linking reagent
SO Ketsueki to Myakkan (1986), 17(4), 385-8
CODEN: KTMYA3; ISSN: 0386-9717
AU Ariga, Toyohiko; Oshiba, Susumu; Hawiger, Jack
PY 1986
AB **Antigen**-antibody complexes or aggregated-IgG caused human platelet aggregation and release reaction. Although presence of **Fc** receptor on the platelet surface is obvious so far, the entity of **Fc** receptor has not been elucidated. The authors have tried to isolate **Fc** receptor by using photosensitive **heterobifunctional** cross-linking reagents. Human platelets were obtained from platelet-rich plasma through an albumin cushion and a Sepharose 2B column. 125I-labeled platelet and Me azidobenzoimidate-coupled IgG (MABI-IgG) were mixed and bound to each other, and then excess mols. of MABI-IgG were washed away gently at 4.degree.. These processes were carried out in a dark room. The platelets which bound with MABI-IgG at the sites of **Fc** receptor were then exposed to uv light, thereby platelet and IgG were cross-linked covalently. Comparing the compns. of 125I-labeled proteins between IgG-cross-linked and non-cross-linked platelets, after these platelets were solubilized with SDS and urea, the cross-linked platelets showed, in SDS-PAGE, giant mols. (the **Fc** receptor bound to IgG via the cross-linking reagent) which barely entered the gel, and also showed disappearance of a protein having mol. wt. of 255,000. This protein was assumed to be the **Fc** receptor. Taking the mol. size and its rate of iodination (.apprx.13%) into consideration, the receptor protein is localized inside of the membrane, representing a subtle expression on the surface.

L22 ANSWER 36 OF 39 CA COPYRIGHT 1996 ACS DUPLICATE 36

AN 101:88579 CA
TI Preparation and some properties of dimeric rabbit IgG antibody
SO Mol. Immunol. (1984), 21(7), 641-5
CODEN: MOIMD5; ISSN: 0161-5890
AU Mota, Gabriela; Sjoequist, J.; Ghetie, V.
PY 1984
AB By reacting rabbit IgG with the fragment AB of protein A from Staphylococcus aureus (mol. wt. 14,000) an IgG dimer was formed with an approx. mol. wt. of 320,000 and a molar compn. of IgG2-AB1. A hybrid dimer with **dual specificity** consisting of IgG anti-sheep red blood cells/AB/IgG anti-bovine red blood cells was also obtained by reacting successively both rabbit antibodies with the AB fragment. The immunol. properties (affinity for **antigen**, complement activation and binding to **Fc** receptors) of the dimeric IgG were investigated in comparison with monomeric rabbit IgG and a tetrameric IgG obtained by reaction with protein A, (IgG2-protein A1)2. The affinity const. for **antigen** for the IgG2-AB1 complex was 30-fold that of noncomplexed IgG, and 60-fold for (IgG2-protein A1)2. The dimeric structure also enhanced complement activation.

L22 ANSWER 37 OF 39 CA COPYRIGHT 1996 ACS DUPLICATE 37
AN 97:203128 CA
TI Antibody-mediated targeting of liposomes
SO NATO Adv. Study Inst. Ser., Ser. A (1982), 47(Targeting Drugs), 185-202
CODEN: NASSDK; ISSN: 0161-0449
AU Weinstein, John N.; Leserman, Lee D.; Henkart, Pierre A.; Blumenthal, Robert
PY 1982
AB A no. of permutations on the use of antibody and **antigen** for targeting liposomes in vitro were studied. Liposomes with lipid bearing the dinitrophenyl (DNP) hapten bound specifically to cells in 3 exptl. configurations: (i) with TNP-modified human peripheral blood lymphocytes as targets and sheep IgG anti-TNP as crosslinking agent; (ii) with murine myeloma MOPC 315 cells (which bear an IgA with high affinity for nitrophenyl haptens) as target, using endogenous surface Ig on the cells as a point of attachment; (iii) with **Fc**-receptor bearing cells as targets and rabbit anti-TNP as an opsonizing agent. The interactions were monitored by encapsulating carboxyfluorescein and/or methotrexate (I) [59-05-2] in the liposomes, and in some expts. by incorporating 14C-dipalmitoylphosphatidylcholine or a fluorescent marker in the lipid. In each study large nos. of liposomes could be bound specifically to the target cells, but they were internalized in significant nos. only in (iii) when cells capable of **Fc** -mediated endocytosis were used. The most striking internalization was found with the mouse macrophage line P388D1. Encapsulated I had a several-fold greater effect on the metab. of P388D1 (as assayed by 3H-deoxyuridine uptake) than did an equiv. amt. of I free in soln. This finding indicated that an appropriately chosen drug can escape the phagosomal system to exert its effect in the cytoplasm. Ig was coupled to liposomal phosphatidylethanolamine covalently, or to the

liposome through covalently coupled Staphylococcus aureus protein A. Coupling is achieved with the **heterobifunctional** agent N-succinimidyl 3-(2-pyridyldithio)propionate. This method of coupling results in minimal aggregation and little leakage of vesicle contents. Liposomes bearing covalently coupled monoclonal antibody bind with high specificity to cells with the corresponding determinants.

L22 ANSWER 38 OF 39 CA COPYRIGHT 1996 ACS DUPLICATE 38
AN 78:14332 CA
TI Preparation of hybrid antibodies and their use in electron-microscopic immunocytochemistry
SO Vop. Med. Khim. (1972), 18(5), 461-7
CODEN: VMDKAM
AU Dorfman, N. A.
PY 1972
AB Purified antibodies to ferritin and mouse .alpha.-globulin isolated by immunosorbtion were treated with pepsin to eliminate the **Fc** fragment. F(ab)2 fragments were sepd. using Sephadex G-150 gel filtration and reduced with .beta.-mercaptoethanol. The reduced fragments were recombined to produce **hybrid antibodies** which were purified on a column of Sephadex G-100 where Fab fragments were sepd. Quant. pptn. showed that the mixt. of F(ab)2 fragments was composed of 53% antiferritin, 44% of hybrid, and 3% antiglobulins F(ab)2 fragments. After addnl. purification, the **hybrid antibodies** were used for the detn. of H-2.alpha. **antigens** location on the surface of leukosis cells. The accuracy of immunocytochem. anal. was higher with **hybrid antibodies** than with ferritin and antibody conjugates.

L22 ANSWER 39 OF 39 CA COPYRIGHT 1996 ACS DUPLICATE 39
AN 76:44507 CA
TI Reverse immune cytoadherence. Technique for the detection of surface-associated .gamma.-globulins on lymphoid cells
SO J. Immunol. Methods (1971), 1(1), 1-17
CODEN: JIMMBG
AU Paraskevas, F.; Lee, S. T.; Orr, K. B.; Israels, L. G.
PY 1971
AB A mixed rosette technique was developed, using 2 **hybrid antibodies**, one specific for mouse .gamma.G (7S .gamma.2a) with an anti-ferritin site and the other for .gamma.F (7S .gamma.1) with an anti-egg albumin site. Ferritin-coated bacteria and egg albumin-coated sheep red cells were used for indicator systems. Most rosette-forming cells in mouse spleen formed mixed rosettes, indicating both .gamma.G and .gamma.F surface globulins and pluripotentiality. Inhibition of rosette formation demonstrated the existence of a surface component (**Fc** receptor) binding the **Fc** of antibodies in **antigen**-antibody complexes of rabbit and mouse antibodies as well as **Fc** from papain-digested, purified rabbit antibodies. Antilymphocyte serum (ALS) blocked the formation of 30-40% of the rosette-forming mouse spleen cells. This block was obsd. at very high dilns. of ALS and

was achieved by the synergistic effect of the 7S and 19S fractions when both were present at certain optimal concns.

FILE 'BIOSIS' ENTERED AT 15:35:02 ON 02 APR 96
COPYRIGHT (C) 1996 BIOSIS(R)

FILE 'MEDLINE' ENTERED AT 15:35:02 ON 02 APR 96

FILE 'EMBASE' ENTERED AT 15:35:02 ON 02 APR 96
COPYRIGHT (C) 1996 Elsevier Science B.V. All rights reserved.

FILE 'LIFESCI' ENTERED AT 15:35:02 ON 02 APR 96
COPYRIGHT (C) 1996 Cambridge Scientific Abstracts (CSA)

FILE 'CANCERLIT' ENTERED AT 15:35:02 ON 02 APR 96

FILE 'BIOTECHDS' ENTERED AT 15:35:02 ON 02 APR 96
COPYRIGHT (C) 1996 DERWENT INFORMATION LTD

FILE 'WPIDS' ENTERED AT 15:35:02 ON 02 APR 96
COPYRIGHT (C) 1996 DERWENT INFORMATION LTD

FILE 'DISSABS' ENTERED AT 15:35:02 ON 02 APR 96
COPYRIGHT (C) 1996 University Microfilms, Inc.

FILE 'CONFSCI' ENTERED AT 15:35:02 ON 02 APR 96
COPYRIGHT (C) 1996 Cambridge Scientific Abstracts (CSA)

FILE 'SCISEARCH' ENTERED AT 15:35:02 ON 02 APR 96
COPYRIGHT (C) 1996 Institute for Scientific Information (ISI) (R)

=> s ((bispecif? or bi specif? or hybrid)(w)antibod? or heterobifunct? or hetero(w)(bifunct? or bi funct? or conjug?) or heteroconjug? or dual? specif? or quadrom?)(l)(fc? or cd16 or cd 16)
L23 80 FILE BIOSIS
L24 87 FILE MEDLINE
L25 84 FILE EMBASE
L26 37 FILE LIFESCI
L27 68 FILE CANCERLIT
L28 24 FILE BIOTECHDS
L29 8 FILE WPIDS
L30 8 FILE DISSABS
L31 1 FILE CONFSCI
L32 57 FILE SCISEARCH

TOTAL FOR ALL FILES
L33 454 ((BISPECIF? OR BI SPECIF? OR HYBRID)(W) ANTIBOD? OR HETERO BIFUNCT? OR HETERO(W)(BIFUNCT? OR BI FUNCT? OR CONJUG?) OR HETEROCONJUG? OR DUAL? SPECIF? OR QUADROM?)(L)(FC? OR CD16 OR CD 16)

```
=> s ran=,1994
ENTER LOGIC EXPRESSION, QUERY NAME, OR (END):133
L34          62 FILE BIOSIS
L35          70 FILE MEDLINE
L36          69 FILE EMBASE
L37          30 FILE LIFESCI
L38          59 FILE CANCERLIT
L39          24 FILE BIOTECHDS
L40           7 FILE WPIDS
L41           7 FILE DISSABS
L42           1 FILE CONFSCI
L43          45 FILE SCISEARCH

TOTAL FOR ALL FILES
L44         374 L33

=> s l44(l)antigen?
L45          25 FILE BIOSIS
L46          31 FILE MEDLINE
L47          30 FILE EMBASE
L48          11 FILE LIFESCI
L49          27 FILE CANCERLIT
L50          11 FILE BIOTECHDS
L51           6 FILE WPIDS
L52           4 FILE DISSABS
L53           0 FILE CONFSCI
L54          15 FILE SCISEARCH

TOTAL FOR ALL FILES
L55         160 L44(L) ANTIGEN?

=> dup rem l55
PROCESSING COMPLETED FOR L55
L56          68 DUP REM L55 (92 DUPLICATES REMOVED)

=> d 1-68 bib abs; fil ca; e ring d/au

L56  ANSWER 1 OF 68  DISSABS COPYRIGHT 1996 UMI
AN   94:37070  DISSABS   Order Number: AAR9424422
TI   IN VITRO AND IN VIVO TUMOUR CELL LYSIS USING THE TUMOUR ASSOCIATED
     ANTIGENS PLACENTAL ALKALINE PHOSPHATASE AND GERM CELL ALKALINE
     PHOSPHATASE AS TARGETS FOR IMMUNOTHERAPY
AU   SMANS, KARINE ALFONSINE [PH.D.]; DE BROE, MARC E. [adviser];
     HOYLAERTS, MARC F. [adviser]; MOENS, LUC [adviser]
CS   UNIVERSITAIRE INSTELLING ANTWERPEN (BELGIUM) (0314)
SO   Dissertation Abstracts International, (1994) Vol. 55, No. 4B, p.
     1370. Order No.: AAR9424422. 256 pages.
DT   Dissertation
FS   DAI
LA   English
AB        In this thesis both in vitro and in vivo tumour cell lysis were
     studied using the tumour associated antigens PLAP and GCAP
     as a target for immunotherapy with anti-(T cell) anti-(tumour cell)
```

bispecific antibodies. As a model for this study the mouse fibroblast cell line MO$\sb4$ which produces invasive sarcomas in the C$\sb3$H-mouse, was used after transfection with PLAP or GCAP. An assay was developed and validated in which a gradual dose dependent tumour cell lysis could be determined over several days by measurements of the PLAP/GCAP activity on residual non-lysed tumour cells in control cultures versus cultures treated with the anti-PLAP anti-CD3 antibody conjugate 7E8-OKT3. The lysis measured already at very low doses of 7E8-OKT3 appeared to be largely CD3-mediated, while lysis by NK/K cells through binding of 7E8-OKT3 to **Fc** receptors (ADCC) was of minor importance. Furthermore, it was found that the targeting of T cells to tumour cells by means of a bispecific anti-(T cell) anti-(tumour cell) antibody conjugate, with subsequent lysis of the targeted tumour cell, is the result of a complex interaction between non-lytic monocytes and T cells, during which event the former cells seemed to activate CD4$\sp+$ T cells. The subsequently released cytokine by the CD4$\sp+$ T cells then activate the CD8$\sp+$ T-cell population, generating targetable cytotoxicity.

For the in vivo studies an immunocompetent mouse model was developed, based on GCAP transgenic mice in which PLAP and GCAP positive solid tumours could be grown. A bispecific anti-(PLAP/GCAP) anti-(mouseCD3) antibody 7E8 x 7D6 was constructed and characterized and administered either systemically or locally to tumour bearing mice. Both strategies were capable of specifically eliminating GCAP positive tumour cells from the tumour. However, an outgrowth of the few GCAP negative tumour cells originally present in the tumour inoculum occurred for the systemic therapy and to a lesser extent for the local therapy where higher numbers of T cells had localized in the tumour. This suggested that the immunotherapy, although capable of specifically eliminating GCAP positive tumour cells, could possibly still be improved by increasing the T cell localization in the tumour and by the use of several tumour markers at once, this to prevent the outgrowth of a tumour lacking the targeted marker.

L56 ANSWER 2 OF 68 BIOTECHDS COPYRIGHT 1996 DERWENT INFORMATION LTD
AN 94-12217 BIOTECHDS
TI Production of hybridoma cell lines producing anti-CD16 antibodies; tetradoma construction, bispecific antibody production and use in e.g. Hodgkin disease therapy
PA Biotest-Pharma
PI DE 4337197 25 Aug 1994
AI DE 93-4337197 30 Oct 1993
PRAI DE 93-4337197 30 Oct 1993
DT Patent
LA German
OS WPI: 94-256364 [32]
AN 94-12217 BIOTECHDS
AB A process is claimed for the selective production of hybridoma cell lines which produce an IgG1 mouse monoclonal antibody (MAb) directed against the human **CD16 antigen** which has a high capacity for inducing natural killer cell

(NKC)-dependent cytotoxicity. The process involves: co-culturing hybridoma cells producing anti-**CD16** MAbs together with unstimulated human NK cells; monitoring the mortality rate of the hybridoma cells; and selecting the hybridoma cells with the highest mortality rate. Also claimed is hybridoma cell line A9 (DSM ACC 2148) which produces a monoclonal antibody of subclass IgG1-lambda, is directed against human **CD16 antigen**, has a high capacity for inducing NKC-dependent cytotoxicity, and binds to a **CD16** epitope separate from that recognized by MAb 3G8. Also claimed is a process for producing bispecific MAbs by fusing hybridomas cells producing anti-CD30 MAb to obtain a tetradoma, and isolating and purifying the resulting **bispecific antibodies**. The **bispecific antibodies** are useful for inducing NKC-mediated cytotoxicity against human tumor cells to cause regression of established tumors, especially Hodgkin disease. (14pp)

L56 ANSWER 3 OF 68 BIOTECHDS COPYRIGHT 1996 DERWENT INFORMATION LTD
AN 94-10426 BIOTECHDS
TI Multivalent multispecific immunoglobulin protein; Fc receptor, tumor marker, carcinoembryonic antigen, HIV virus-1, hormone, cytokine, tumor necrosis factor, etc. multispecific antibody engineering and phage display
PA Cambridge-Antibody-Technol.; Med.Res.Counc.
PI WO 9413804 23 Jun 1994
AI WO 93-GB2492 3 Dec 1993
PRAI GB 93-19969 22 Sep 1993; GB 92-25453 4 Dec 1992
DT Patent
LA English
OS WPI: 94-217880 [26]
AN 94-10426 BIOTECHDS
AB A new chimeric antibody (Ab) has a 1st domain containing a binding region of an Ig heavy chain variable (V) region, and a 2nd domain with a binding region of an Ig light chain V region, linked but incapable of associating to form an antigen binding site (ABS). The domains may be linked directly or via a peptide linker. The binding regions bind the same or different epitopes. A peptide tag, signal peptide or filamentous phage surface component domain, e.g. GIII may be included. A dimer of different polypeptides with binding regions associated to form an ABS is also new. DNA encoding the protein is also new, and may also encode an RNA splice site, a suppressible stop codon, or an in vivo or in vitro recombination site between 1st domain and 2nd domain regions. A new vector contains the DNA and sequences for secretion and phage display in a recombinant host. The protein may be recovered from the culture supernatant, periplasm or via protein renaturation. The Ab binds e.g. an Fc receptor, tumor marker, carcinoembryonic antigen, HIV virus-1, hormone, cytokine, tumor necrosis factor, Ab or idiotope. (180pp)

L56 ANSWER 4 OF 68 WPIDS COPYRIGHT 1996 DERWENT INFORMATION LTD
AN 94-200784 [25] WPIDS
DNN N94-157939 DNC C94-091771

TI New tetrameric, bifunctional immunological complexes - contain two different antibodies of first species linked by two antibodies of second species, useful in immunoassay, blood typing, treatment of cancer and infections.
DC B04 D16 S03
IN LANSDORP, P M
PA (TETR-N) TETRAMERICS BIOTECHNOLOGY INC
CYC 1
PI CA 1329545 C 940517 (9425)* 31 pp
ADT CA 1329545 C CA 87-535725 870428
PRAI CA 87-535725 870428
AN 94-200784 [25] WPIDS
AB CA 1329545 C UPAB: 940810
New bifunctional immunological complex (A) contains two different antibodies (Ab1, Ab2) from a first species conjugated, to form a cyclic tetramer with two antibodies (Ab3) of a second species directed against the **Fc** fragments of Ab1 and Ab2. Also new are similar complexes (A') in which Ab1 and Ab2 are homologous.
USE/ADVANTAGE - (A) can be used as immunoassay reagents to detect Ab1/Ab2 specific Ag, where one Fab fragment is directed against an erythrocyte surface membrane **antigen** and the other against HLA-DR antibodies they can be used to determine blood groups, partic. by agglutination reaction. (A) can also be used to treat cancer and infectious diseases, i.e. they can direct cytotoxic or other cpds. to target cells. (A') are also useful for determining blood gps. partic. in a dilution of 1:1000 as Coombs reagent or in indirect agglutination. (A) are very stable and can be prepd. without time-consuming prepn. of hybrid hybridomas (required to produce known **bispecific antibodies**). Complex prepn. is very simple and rapid (make efficient than chemical labelling of antibodies) and Ab3 are specific that non-purified antibodies can be reacted.
Dwg.1c/3

L56 ANSWER 5 OF 68 BIOSIS COPYRIGHT 1996 BIOSIS DUPLICATE 2
AN 94:177754 BIOSIS
DN 97190754
TI Targeting HIV-1 to Fc-gamma-R on human phagocytes via bispecific antibodies reduces infectivity of HIV-1 to T cells.
AU Howell A L; Guyre P M; You K-S; Fanger M W
CS Dep. Microbiol., HB 7550, Dartmouth Medical Sch., Hanover, NH 03755-3842, USA
SO Journal of Leukocyte Biology 55 (3). 1994. 385-391. ISSN: 0741-5400
LA English
AB In addition to CD4, the primary receptor to which the human immunodeficiency virus type 1 (HIV-1) binds, mononuclear phagocytes (monocytes) express three classes of **Fc** receptors for immunoglobulin G (**Fc**-gamma-R). We have previously shown that infection of monocytes by HIV-1 is inhibited when **bispecific antibodies** (BsAbs) are used to target the virus to either the type I, type II, or type III **Fc** -gamma-R on these cells. Infection of monocytes was not inhibited when HIV-1 was targeted to either human leukocyte **antigen**

class I or CD33. We have extended these studies to examine the ability of BsAbs plus polymorphonuclear leukocytes (neutrophils, PMNs) and monocytes to reduce infectivity of HIV-1 to cells from the human T cell lymphoma line, H9. The production of HIV-1 following interaction of virus with BsAb and phagocytes was determined in an indicator cell assay by mixing BsAb, HIV-1, and phagocytes with uninfected H9 cells. Productive infection of H9 cells was quantitated on subsequent days by measuring p24 gag **antigen** levels in supernatants by enzyme-linked immunosorbent assay. Our findings show that the addition of interferon-gamma-activated PMNs or monocytes to cultures of HIV-1 plus H9 cells in the absence of BsAb results in a marked reduction in p24 levels equivalent to 85 to 90% of control levels. With the combination of BsAb (anti-**Fc**-gamma-RI times anti-gp120) plus IFN-gamma-activated phagocytes, levels of p24 in H9 cultures were below those at culture initiation. These findings demonstrate that IFN-gamma-activated phagocytes can affect the natural course of HIV-1 infection of T cells, a finding of potential clinical importance.

L56 ANSWER 6 OF 68 BIOSIS COPYRIGHT 1996 BIOSIS DUPLICATE 3
AN 94:226124 BIOSIS
DN 97239124
TI A bifunctional murine::human chimeric antibody with one antigen-binding arm replaced by bacterial beta-lactamase.
AU De Sutter K; Fiers W
CS Lab. Mol. Biol., Gent Univ., K.L. Ledeganckstraat 35, B-9000 Gent, BEL
SO Molecular Immunology 31 (4). 1994. 261-267. ISSN: 0161-5890
LA English
AB We here report the genetic engineering of a murine::human chimeric antibody-directed against the tumor marker human placental alkaline phosphatase-in which one **antigen**-binding arm (Fab) has been replaced by Escherichia coli beta-lactamase (Bla). A mutated Bla gene in which the termination codon had been replaced by GAG, was fused in-phase to the cDNA sequence encoding the hinge region, CH2 and CH3 of the human IgG3 heavy chain. The resulting BlaHG3f fusion gene was placed under control of the Simian Virus 40 late promoter, and transiently expressed in COS-1 cells together with the genes encoding the murine light and murine:: human chimeric heavy chains. Approximately 200 ng/ml of correctly assembled bifunctional antibody-Bla immunoconjugates were detected in the culture supernatant. This observation indicates that Bla (with its own leader peptide) can efficiently direct secretion into the culture medium of adventitious sequences fused at its C-terminus. Furthermore, the assembly in the **Fc** region was not affected by steric hindrance due to a Bla moiety and an Fab arm in close proximity. The antibody-Bla immunoconjugate could be of therapeutic value for the activation of cephalosporin-based anti-cancer prodrugs at the tumor site. Moreover, the expression strategy adopted here is particularly suitable for a quick and convenient analysis of newly designed gene products in which the Bla moiety has been replaced by other enzymes or by **antigen**-binding fragments in order to engineer **bispecific antibodies.**

L56 ANSWER 7 OF 68 BIOTECHDS COPYRIGHT 1996 DERWENT INFORMATION LTD
AN 94-14080 BIOTECHDS
TI Bivalent and bispecific diabodies fr colorectal tumor targeting; (conference abstract)
AU Cochet O; Prospero T; Chester K A; Teillaud J L; Winter G; Hawkins R E
CS Cent.Protein-Eng.Cambridge
LO Centre for Protein Engineering, Hills Road, Cambridge, CB2 2QH, UK.
SO J.Cell.Biochem.; (1994) Suppl.18D, 211
CODEN: JCEBD5
DT Journal
LA English
AN 94-14080 BIOTECHDS
AB A bispecific anti-carcinoembryonic **antigen** (anti-CEA) and anti-**Fc**-gamma receptor-III (anti-**CD16**) diabody was constructed. The antibody variable genes were derived from hybridoma 3G8 (anti-**CD16**) and from a phage display gene bank (anti-CEA) and were formatted initially as single chain antibody Fv fragments. They were converted to diabody format by shortening the sc-linker from 15 to 5 amino acids (GGGGS), which prevented the intrachain pairing of VH and VL domains, favoring dimer formation. The diabodies bound to their target **antigens** (ELISA and FACS analysis). BIACORE measurements showed that the bivalent anti-CEA diabody had an affinity of about 3 nM, which was the same as the initial scFv. The affinity of the bivalent anti-**CD16** diabody was about 10 nM. The VL domains of the 2 diabodies were exchanged to create 2 complementary proteins each comprising 2 antibody V domains. The 2 proteins were expressed in Escherichia coli from a plasmid pUC119 vector. The recombinant **bispecific antibodies** bound to CEA and **CD16** with similar kinetics to the parent diabodies. Bispecific diabodies are promising for target cell destruction using natural cellular effector mechanisms. (0 ref)

L56 ANSWER 8 OF 68 LIFESCI COPYRIGHT 1996 CSA
AN 94:84677 LIFESCI
TI Promotion of natural killer cell growth in vitro by bispecific (anti-CD3 x anti-CD16) antibodies
AU Malygin, A.M.; Somersalo, K.*; Timonen, T.
CS Dep. Pathol., P.O. Box 21, (Haartmaninkatu 3), Univ. Helsinki SF-00014 Helsinki, Finland
SO IMMUNOLOGY, (1994) vol. 81, no. 1, pp. 92-95.
ISSN: 0019-2805.
DT Journal
FS F
LA English
SL English
AB Bispecific heteroconjugated F(ab') sub(2) fragments were prepared from pepsin-digested monoclonal OKT3 (anti-CD3) and 3G8 (anti-CD16) with 5,5'-dithiodbis-(2-nitrobenzoic acid). When these bispecific antibodies (BsA) were added to peripheral blood lymphocyte (PBL) cultures with 100 U/ml human recombinant interleukin-2 (rIL-2)

preferable growth of natural killer cells occurred. The results show that bispecific (anti-CD3 x anti-CD16) F(ab') sub(2) fragments are strongly immunomodulatory by inducing the killing of T cells by CD16 super(+) cells.

L56 ANSWER 9 OF 68 BIOSIS COPYRIGHT 1996 BIOSIS DUPLICATE 4
AN 94:535262 BIOSIS
DN 97548262
TI A bispecific monoclonal antibody with versatile adaptor mediating different cytotoxicities.
AU Wang L-M; Wan W-H; Lu L-J; et al
CS Dep. Biochem., Beijing Inst. Cancer Res., Beijing 100034, CHN
SO Zhonghua Zhongliu Zazhi 16 (2). 1994. 83-87. ISSN: 0253-3758
LA Chinese
AB Bispecific monoclonal antibody (bsMAb), secreted by hybrid-hybridoma, has an intact 1g molecule construction with dual **antigen** binding specificities. bsMAb has several advantages over conventional conjugate in tumor targeting therapy: (1) The damage to both antibody and tumoricidal agents due to chemical crosslinking can be avoided. (2) The **antigen** modulation can be decreased due to monovalence binding of bsMAb with target **antigen**. (3) The binding affinity between **Fc** fragment of bsMAb with heterogenous heavy chains and **Fc** receptor is decreased, so that nonspecific distribution and side effects can be reduced. (4) The bsMAb can pretarget to tumor site, increasing the T/NT ratio and cytotoxicity. In the present study, A bsMAb with versatile adaptor was designed. In this bsMAb, one arm could react with gastric cancer - associated **antigen**, and the other with a hapten TNP. The bsMAb could mediate different tumoricidal agents crosslinked to TNP. A variant hybridoma 3H11-HAT-s secreting McAb against gastric cancer was fused with spleen cells of mice immunized with KLH-TNP. After screening and subcloning, 10 hybrid-hybridomas were obtained, which secreted antibodies against both gastric cancer target cells BGC823 and BSA-TNP. By using special bridge method, only hybrid-hybridomas 6A3(gamma-2b, mu), and 1G7(gamma 2b, gamma 2b) were confirmed to secrete bsMAb. Further experiments showed that the bsMAb 6A3 and 1G7 could mediate different cytotoxicities, for example, Ricin-TNP, MMC-HSA-TNP and ADM-BSA-TNP. This system is useful for evaluating different tumoricidal agents in bsMAb targeting therapy, and has potential value in clinics. In addition, the significance of special bridge method for confirmation of bsMAb was emphasized. In other word, supernatants secreted by hybrid-hybridomas with **dual specificities** may not contain bsMAb. It is generally believed that mu chain could not combine with gamma chain to form bsMAb. However, in our particular case, hybrid-hybridoma 6A3 does secrete bsMAb with mu chain and gamma 2b, as confirmed by subclass analysis, bridge method and cytotoxicities in vitro.

L56 ANSWER 10 OF 68 MEDLINE DUPLICATE 5
AN 94348765 MEDLINE
TI Production and use of anti-FcR bispecific antibodies.
AU Fanger M W; Graziano R F; Guyre P M
CS Department of Microbiology, Dartmouth Medical School, Lebanon, New

Hampshire 03756..
SO IMMUNOMETHODS, (1994 Feb) 4 (1) 72-81. Ref: 91
Journal code: B3R. ISSN: 1058-6687.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, ACADEMIC)
LA English
FS Priority Journals
EM 9412
AB **Bispecific antibodies** (BsAb) are antibodies with two different specificities. BsAb composed of anti-**Fc** gamma R Ab linked to anti-target Ab have been useful in exploring the function of the three classes of human **Fc** gamma R. In addition, BsAb have been developed as new agents for immunotherapy which can join together different molecules or cells. In directed or redirected cytotoxicity, BsAb that bind both to target cells (pathogens or tumors) and to triggering molecules on leukocytes such as **Fc** gamma R are used to focus normal cellular immune defense mechanisms specifically to the tumor cell or infectious agent. Limited clinical trials have demonstrated little toxicity and promising responses. This ability to redirect normal cytotoxic mechanisms to kill tumors, infectious agents, or infected cells makes BsAb powerful new therapeutic tools. In addition, BsAb are being used to target other appropriate molecules to **Fc** gamma R, including **antigens** as vaccine adjuvants and immune complexes. This review focuses on BsAb in which one specificity is directed to **Fc** gamma R on human leukocytes. It considers applications of these reagents and discusses the progress toward an understanding of the construction and use of BsAb in therapy.

L56 ANSWER 11 OF 68 CANCERLIT
AN 94697527 CANCERLIT
TI Bispecific antibodies recruiting cellular effectors (Meeting abstract).
AU Thielemans K
CS Lab. of Physiology-Immunology, Vrije Universiteit Brussel, B-1090 Brussels, Belgium
SO Non-serial, (1993). EACR-12: 12th Biennial Meeting of the European Association for Cancer Research. April 4-7, 1993, Brussels, Belgium, 1993.
DT (MEETING ABSTRACT)
FS ICDB
LA English
EM 9411
AB The idea to target T lymphocytes toward tumor cells has been tested extensively and successfully by many laboratories. Our laboratory has evaluated this attractive approach in vivo. We have developed **bispecific antibodies** by hybrid-hybridoma technique, with **dual specificity**: the murine CD3 complex and the idiotype (tumor specific **antigen**) of the surface expressed IgM on two murine B-cell lymphomas. Since the

primary goal of our experiments was to evaluate the therapeutic potential of this approach in vivo, we have invested great efforts in the generation of proper control reagents. Using the aggressive 38C13 lymphoma of C3H origin, we showed that **bispecific antibodies** could cure a significant number of tumor-bearing animals. Animals with even a lower tumor burden were not protected with any of the control antibodies (bi- or univalent class-matched anti-idiotype mAb, bi- or univalent anti-CD3 mAb). Tumor-bearing C3H/HeJ mice (not able to perform **FcR**-dependent ADCC) could also be successfully treated. Animals in which we had induced allo-sensitized effector T cells showed a much higher cure rate in comparison with the experiments in untreated animals. Further evidence that the therapeutic effect was T-cell-mediated and dependent on the bridge formation between tumor cells and T cells, was obtained by using a tumor-**antigen** negative variant of the 38C13 tumor line and by using a mixture of a T-cell binding/nontumor-cell binding antibody plus a tumor-cell binding/nonT-cell binding antibody. Similar results were obtained with the BCL1 lymphoma model in BALB/c mice. The efficacy of the **bispecific antibodies** was dependent on both tumor load and dose. Repeated injections of **bispecific antibodies** could cure more mice than a single injection. Treatment of tumor-bearing mice in which either CD4+, CD8+ or both subsets had been depleted, clearly indicated the T-cell-mediated effect and the contribution of both subsets to the tumor eradicating mechanism. Taken together, our data indicate that it is indeed possible to recruit and focus T cells against a given target in vivo. Ongoing experiments explore the T-cell-mediated mechanism and the potential improvement of our results by combining two **bispecific antibodies** which will activate the T cells at the tumor site via CD3 and CD28.

L56 ANSWER 12 OF 68 BIOSIS COPYRIGHT 1996 BIOSIS DUPLICATE 6
AN 93:435531 BIOSIS
DN BA96:90156
TI INVOLVEMENT OF THE HIGH-AFFINITY RECEPTOR FOR IGG FC-GAMMA-RI CD64 IN ENHANCED TUMOR CELL CYTOTOXICITY OF NEUTROPHILS DURING GRANULOCYTE COLONY-STIMULATING FACTOR THERAPY.
AU VALERIUS T; REPP R; DE WIT T P M; BERTHOLD S; PLATZER E; KALDEN J R; GRAMATZKI M; VAN DE WINKEL J G J
CS DIV. HEMATOL/ONCOL., DEP. MED. III, UNIV. ERLANGEN-NUERNBERG, KRANKENHAUSSTRASSE 12, 8520 ERLANGEN, GER.
SO BLOOD 82 (3). 1993. 931-939. CODEN: BLOOAW ISSN: 0006-4971
LA English
AB Three different classes of **Fc** receptors for IgG (**Fc** .gamma.R) are currently distinguished in humans, of which polymorphonuclear phagocytes (PMN) normally express both low-affinity receptor classes - **Fc**.gamma.RII (CD32) and **Fc** .gamma.RIII (**CD16**). During therapy with granulocyte colony-stimulating factor (G-CSF), neutrophils from patients with various malignancies and different hematologic disorders were found to additionally express high levels of the receptor with high affinity for IgG (**Fc**.gamma.RI; CD64). For these patients,

the relative fluorescence intensity (rFI) for **Fc**.gamma.RI was 5.3 (range, 1.7 to 10.3; n = 19), compared with 1.0 (range, 1.0 to 1.1; n = 8) for healthy donors. The expression of **FC** .gamma.RI during GCSF therapy could be confirmed by using a panel of six CD64-specific antibodies, and by showing mRNA for **Fc** .gamma.RI. So far, three genes for **Fc**.gamma.RI have been identified, encoding four distinct transcription products. By reverse transcriptase-polymerase chain reaction technology, transcripts for both membrane-associated isoforms (hFc.gamma.RIa and hFc.gamma.RIb2) could be detected. The functional activity of **Fc**.gamma.RI on PMN during G-CSF therapy was shown by measuring binding of monomeric human IgG and antibody-dependent cellular cytotoxicity (ADCC). Thus, **Fc**.gamma.RI-positive neutrophils displayed enhanced ADCC activity to glioma (A1207), squamous cell (A431), and ovarian (SK-ov3) carcinoma cell lines. The involvement of **Fc** .gamma.RI in this increased cytotoxic activity was shown by blocking **Fc**.gamma. receptors with monoclonal antibodies, and by using F(ab')2 .times. F(ab')2-**bispecific antibodies** with specificities against tumor-related **antigens** and **Fc**.gamma.RI, resulting in solely **Fc** .gamma.RI-mediated cytotoxicity. Therapeutically, this additional **Fc** receptor on PMN may increase the efficacy of experimental antibody therapy.

L56 ANSWER 13 OF 68 BIOSIS COPYRIGHT 1996 BIOSIS DUPLICATE 7
AN 94:112896 BIOSIS
DN 97125896
TI Immunohistochemical detection of c-erbB-2 expression by neoplastic human tissue using monospecific and bispecific monoclonal antibodies.
AU Garcia De Palazzo I; Klein-Szanto A; Weiner L M
CS Fox Chase Cancer Center, 7701 Burholme Ave., Philadelphia, PA 19111, USA
SO International Journal of Biological Markers 8 (4). 1993. 233-239. ISSN: 0393-6155
LA English
AB Selected murine monoclonal antibodies (M Ab) have been shown to inhibit relevant tumor growth in vitro and in animal models. Recently, **bispecific antibodies** (BsMAb) have been developed which target cytolytic effector cells via one antibody binding site and tumor **antigen** by the other specificity. For example, the BsM Ab 2B1 possesses specificity for c-erb B-2 and **Fc**-gamma-RIII, the low affinity **Fc**-gamma receptor expressed by polymorphonuclear leukocytes (PMN), macrophages and large granular lymphocytes (LGL). The human homologue of the rat neu oncogene, c-erbB-2, has been demonstrated to be amplified in breast, gastrointestinal, lung and ovarian carcinomas. Tumor expression of c-erbB-2 has been shown to be an important prognostic indicator in breast and ovarian carcinomas. The restricted expression of the c-erbB-2 protooncogene product in normal human tissues and the wide distribution of c-erbB-2 expression in such tumors may justify attempts to use an appropriately constructed Bs M Ab in clinical trials. In this report we have addressed this issue by immunohistochemically evaluating the expression of c-erbB-2 oncogene

product in a variety of malignant tumors utilizing 2B1 and the anti-c-erbB-2 monovalent parent of 2B1, 520C9. Among the studied neoplasms, c-erbB-2 expression was detected in 49% of primary carcinomas stained with 520C9 and in 39% of those stained with 2B1. In the group of metastatic tumors, c-erbB-2 oncoprotein was detected in 52% of cases by 520C9 and in 41% by 2B1. Our results indicate that immunocytochemistry using bispecific monoclonal 2B1 is a reliable method for the detection of c-erbB-2 expression, and that this Bs M Ab detects c-erbB-2 expression in tumors nearly as well as its anti-c- erbB-2 monovalent parent antibody.

L56 ANSWER 14 OF 68 EMBASE COPYRIGHT 1996 ELSEVIER SCI. B.V.
AN 93245347 EMBASE
TI Bispecific antibody as a potentiator of tumor cell killing by IL-2-activated lymphocytes.
AU Yagita H.; Ikeda M.; Nitta T.; Sato K.; Okumura K.; Ishii S.
CS Department of Immunology, Juntendo Univ. School of Medicine, 2-1-1 Hongo, Bunkyo-ku, Tokyo 113, Japan
SO GANN MONOGR. CANCER RES., (1993) 40/- (95-106).
ISSN: 0072-0151 CODEN: GANMAX
CY Japan
DT Journal
FS 008 Neurology and Neurosurgery
016 Cancer
026 Immunology, Serology and Transplantation
037 Drug Literature Index
LA English
SL English
AB Lymphokine-activated killer (LAK) cells, which can be readily induced from peripheral blood lymphocytes (PBL) by culturing with interleukin 2 (IL-2) and exhibit broad cytotoxicity against various tumor target cells, have been the subject of clinical trials of adoptive immunotherapy of cancer patients. Such trials throughout the world, however, have resulted in limited success to date. This appears to result largely from the inefficient recognition of various target cells by LAK cells, the mechanisms for which are still unclear. In order to bypass such an obscure step in the LAK cell-mediated cytotoxicity, we used **heteroconjugated** antibodies composed of a monoclonal antibody (mAb) to triggering molecules on LAK cells, such as CD3 and **CD16**, chemically cross-linked with a mAb to a glioma-associated **antigen**. Such **bispecific antibodies** (BsAb) redirected the LAK cell cytotoxicity against glioma cells in a highly specific and effective manner in vitro. The specific targeting therapy using LAK cells in conjunction with BsAb resulted in a striking clinical efficacy in post-operational treatment of glioma patients as compared with the classical LAK therapy.

L56 ANSWER 15 OF 68 DISSABS COPYRIGHT 1996 UMI
AN 94:10446 DISSABS Order Number: AARC325850 (not available for sale by UMI)
TI INTERACTIONS OF ANTIBODY DERIVATIVES WITH TARGET AND EFFECTOR CELL SURFACES (TARGET CELLS, EFFECTOR CELLS)

AU CURNOW, STEPHEN JOHN [PH.D.]
CS UNIVERSITY OF SOUTHAMPTON (UNITED KINGDOM) (5036)
SO Dissertation Abstracts International, (1992) Vol. 55, No. 1C, p. 122. Order No.: AARC325850 (not available for sale by UMI).
DT Dissertation
FS DAI
LA English
AB Bispecific F(ab$\sp\prime\gamma)\sb2$ antibody derivatives, constructed by the disulphide or thioether linking of two Fab$\sp\prime\gamma$ fragments, have been used to link target and effector cells via specific surface molecules. Effects on both target and effector cells were studied, in particular the induction of **antigenic** modulation, changes in intracellular calcium ion concentration ((Ca$\sp{2+}\rbrack\sb{\rm i}$), and apoptosis.

Antigenic modulation, induced by bivalent antibody in the absence of cellular interactions, was observed with surface Ig (sIg) on tumour target cells, but not **Fc**γ receptor (**Fc**γR) I, **Fc**γRII, or **Fc**γRIII on effector cells. **Bispecific antibody** derivatives (functionally univalent for individual cells) did not induce significant **antigenic** modulation upon interaction with only one cell type. Likewise interactions between target and effector cells, induced by F(ab$\sp\prime\gamma)\sb2$ specific for sIg and an **Fc**γR, yielded little modulation of either molecule. However after cell contact was ended by reduction of the disulphide bond between the two Fab$\sp\prime\gamma$, significant internalisation of the now freed sIg and **Fc**γR was seen.

Increases in (Ca$\sp{2+}\rbrack\sb{\rm i}$ were observed upon bivalent or multivalent cross-linking by conventional antibody of sIg, CD37, or CD19 on tumour target cells, CD3 or CD2 on T lymphocytes, and all **Fc**γR on monocytes, granulocytes, and NK cells. Binding of target cells to effector cells, by **bispecific antibodies** linking pairs of the above molecules, induced similar increases in (Ca$\sp{2+}\rbrack\sb{\rm i}$, except for the absence of any signal via the granulcoyte **Fc**γRIII.

Significant target cytotoxicity occurred when **bispecific antibodies** recruited NK cells or monocytes, but not granulocytes, via **Fc**γR. Chelation of intracellular Ca$\sp{2+}$ in the NK cells or monocytes reduced the observed cytotoxicity, suggesting a role for Ca$\sp{2+}$ in activation for lysis.

Apoptosis was shown to occur apparently spontaneously in vitro in one target cell studied, guinea-pig leukaemic lymphocytes, and was seen in NK lysis of susceptible targets. However, cellular cytotoxicity dependent upon antibody--ADCC recruited via either **Fc**γ of IgG or bispecificity of F(ab$\sp\prime\gamma)\sb2$--failed to induce apoptosis.

L56 ANSWER 16 OF 68 BIOTECHDS COPYRIGHT 1996 DERWENT INFORMATION LTD
AN 92-10425 BIOTECHDS
TI Bispecific monoclonal antibody recognizing P-glycoprotein and

HFC-gamma-RIII;
bispecific antibody prepared from tetradoma and useful for cancer therapy, optionally in combination with interleukin-2 and/or macrophage colony stimulating factor

PA Cetus
PI WO 9208802 29 May 1992
AI WO 91-US6949 24 Sep 1991
PRAI US 90-605399 29 Oct 1990
DT Patent
LA English
OS WPI: 92-200186 [24]
AN 92-10425 BIOTECHDS
AB A **bispecific antibody** (BAb) capable of binding to a tumor cell (I) with multidrug-resistance and to a cytotoxic cell **Fc** receptor, especially human **Fc** -gamma-RIII is claimed. The BAb can crosslink a tumor cell and a cytotoxic cell, facilitating tumor lysis. (I) overexpresses P-glycoprotein **antigens**. The BAb is secreted by a hybrid hybridoma (tetradoma), which secretes 2 monoclonal antibodies (MAbs) one specific for (I), and the other specific for a cytotoxic cell **Fc** receptor. The hybridoma secreting the (I)-specific MAb is 15D3 (ATCC HB 10500), while the other is 3G8 (ATCC HB 10501). The following are also claimed: (1) a composition of the BAbs with a suitable adjuvant for use in tumor therapy; (2) the hybrid hybridomas; (3) a method for treating a disease state using BAbs and monitoring progress; (4) a method for detecting fused cells (triomas or tetradomas) by dyeing one fusion partner prior to fusion; (5) an armed effector cell composition comprising cytotoxic cells with BAbs attached, and optionally interleukin-2 and macrophage colony stimulating factor. (61pp)

L56 ANSWER 17 OF 68 BIOTECHDS COPYRIGHT 1996 DERWENT INFORMATION LTD
AN 92-13213 BIOTECHDS
TI Bispecific antibody reacting with phycoerythrin and leukocyte surface antigen;
prepared by fusion of monospecific hybridoma

PA Univ.Leipzig
PI DD 299232 9 Apr 1992
AI DD 89-330162 29 Jun 1989
PRAI DD 89-330162 29 Jun 1989
DT Patent
LA German
OS WPI: 92-293005 [36]
AN 92-13213 BIOTECHDS
AB Production of bispecific, phycoerythrin (I)-binding **hybrid antibodies** (HAb) comprises fusing hybridomas which secrete (I)-specific monoclonal antibodies (MAb1) with hybridomas which produce monoclonal antibodies (MAb2) which bind to the cell surface **antigens** of human leukocytes. The resulting hybrid-hybridomas are cultured and the HAb are recovered conventionally from the culture supernatant or ascites fluid. MAb1 are raised against (I) isolated from red algae, especially R-phycoerythrin (Ia) from Ceramium rubrum produced by the hybridoma

H-BL-PE/34. The MAb2-producing hybridomas are: H-BL-TP3(CD3), -TH4(CD4), -TS8(CD8),-LGL(**CD16**), -TB45R(CD45R), TAC (CD25), Ac/p26 (CD69) or -1a. MAb1-producing hybridomas are prepared by immunizing BALB/c mice with (I), B-lymphocytes are isolated from the spleen and enriched in an **antigen** -specific manner. They are then fused conventionally with X63-Ag8.653 cells. The hybrid-hybridomas are selected by dual wavelength fluorescence in a cell sorter, then grown in vivo, or in vitro. HAb are used with (I) in fluorescence tests for detecting and analyzing the **antigens** to which MAb2 are specific. (8pp)

L56 ANSWER 18 OF 68 BIOTECHDS COPYRIGHT 1996 DERWENT INFORMATION LTD
AN 92-11325 BIOTECHDS
TI Antitumoral molecules which mimic IgE effector functions; recombinant bispecific antibody or chimeric antibody production by expression in Escherichia coli, NSO or CHO cell culture; antibody engineering
PA Cambridge-Antibody-Technol.
PI WO 9211031 9 Jul 1992
AI WO 91-GB2303 23 Dec 1991
PRAI GB 90-27767 21 Dec 1990
DT Patent
LA English
OS WPI: 92-249853 [30]
AN 92-11325 BIOTECHDS
AB A novel molecule (I) which is able to mediate IgE effector mechanisms against a tumor cell expressing a tumor antigen comprises: i. a 1st binding domain, BD1, capable of binding to the antigen; and ii. a 2nd binding domain, BD2, capable of binding to an Fc-epsilon receptor on cells of the immune system. Also claimed are: a. culture medium, ascites or serum containing (I); b. preparation of (I) by inserting DNA sequences encoding BD1 and/or BD2 into vectors containing appropriate regulatory sequences, transforming host cells with the vectors and culturing the hosts to recover expressed BD1 and BD2. BD1 and BD2 are derived from immunoglobulin or are synthetic analogs or part of naturally occurring immunoglobulin domains. (I) is particularly an IgE antibody, or is chimeric, comprising BD1 from an Ig of isotype other than IgE, and BD2 from an IgE. The nucleotide sequences encoding BD1 and BD2 are obtained using the polymerase chain reaction. The host cell is e.g. Escherichia coli, or mouse myeloma cell NSO or CHO. (I) can be used for the destruction of tumor cells and multicellular tumors in host mammals, particularly humans. (31pp)

L56 ANSWER 19 OF 68 BIOSIS COPYRIGHT 1996 BIOSIS DUPLICATE 10
AN 93:6804 BIOSIS
DN BA95:6804
TI ANTITUMOR EFFECTS OF A **BISPECIFIC ANTIBODY** TARGETING CA19-9 **ANTIGEN** AND **CD16**.
AU DE PALAZZO I G; HOLMES M; GERCEL-TAYLOR C; WEINER L M
CS FOX CHASE CANCER CENT., 7701 BURHOLME AVENUE, PHILADELPHIA, PA.

19111, USA.
SO CANCER RES 52 (20). 1992. 5713-5719. CODEN: CNREA8 ISSN: 0008-5472
LA English
AB Bispecific murine monoclonal antibodies that target tumor and **Fc**.gamma.RIII (**CD16**) can promote relevant tumor lysis by large granular lymphocytes. For these antibodies to be clinically useful, their properties should be maintained in vivo, where competing human immunoglobulin, shed target **antigen**, and shed **CD16** may be encountered. At a minimum, **bispecific antibody** antitumor effects should be preserved in whole blood. Furthermore, potentiation of tumor lysis should be reflected by demonstrating the ability of **bispecific antibody**-retargeted effector cells to infiltrate and mediate lysis of organized tumor. If these characteristics are demonstrated, and there is evidence of in vivo efficacy of **bispecific antibody**-based therapy in a relevant animal model, further clinical development of such antibodies would be warranted. In this report the ability of CL158 **bispecific antibody** supernatants to mediate lysis of SW948 tumor growing in monolayer is shown to be preserved in the presence of interleukin 2-activated whole blood. When SW948 cells were grown in vitro as multicellular human tumor spheroids, incubation with interleukin 2-activated lymphocytes (LAK cells) and CL158 led to structural and widespread necrosis. This was dependent on CL158 and resistant to competition by pooled human immunoglobulin or interleukin 2-exposed whole blood. These effects were not promoted by the monospecific antibodies produced by the parent clone of CD158 and were not observed when the IgG2a variant of CA19-9 antibody, which mediates conventional antibody-dependent cellular cytotoxicity, was used instead of its bispecific derivative. To examine the efficacy of **bispecific antibody**-based treatments on in vivo tumor, scid mice bearing early s.c. SW948 xenografts were treated with interleukin 2 for 5 consecutive days, supplemented by three i.v. injections of 107 human LAK cells and various antibodies. Treatment of mice bearing SW948 tumors with LAK cells did not retard tumor growth, but when CL158 was added, significant delays in tumor growth were observed. Tumor growth delay required treatment with both LAK cells and the **bispecific antibody**. Treatment with the IgGa variant of CA19-9 antibody, alone with LAK cells, had no effects on tumor growth. Although the mechanisms of these antitumor effects require further study, it is clear that human LAK cells treatment of animals bearing early, established s.c. tumors is enchanced by the addition of **bispecific antibodies** with relevant binding characteristics. When compared with the IgG2a isotype variant of CA19-9 monoclonal antibody, this **bispecifically antibody** offers the advantages of preservation of activity in physiological conditions, infiltration and disruption of organized tumor in vitro, and antitumor effects in a relevant xenograft model. These characteristics support the continued clinical development of **bispecific antibodies** with specificity for tumor and **Fc** .gamma.RIII.

L56 ANSWER 20 OF 68 LIFESCI COPYRIGHT 1996 CSA
AN 93:66453 LIFESCI
TI Antitumor effects of a **bispecific antibody** targeting CA19-9 **antigen** and **CD16**.
AU Garcia de Palazzo, I.; Holmes, M.; Gercel-Taylor, C.; Weiner, L.M.
CS Fox Chase Cancer Cent., 7701 Burholme Ave., Philadelphia, PA 19111, USA
SO CANCER RES., (1992) vol. 52, no. 20, pp. 5713-5719.
DT Journal
FS F
LA English
SL English
AB Bispecific murine monoclonal antibodies that target tumor and **Fc** gamma RIII (**CD16**) can promote relevant tumor lysis by large granular lymphocytes. For these antibodies to be clinically useful, their properties should be maintained in vivo, where competing human immunoglobulin, shed target **antigen**, and shed **CD16** may be encountered. At a minimum, **bispecific antibody** antitumor effects should be preserved in whole blood. In this report the ability of CL158 **bispecific antibody** supernatants to mediate lysis of SW948 tumor growing in monolayer is shown to be preserved in the presence of interleukin 2-activated whole blood. When SW948 cells were grown in vitro as multicellular human tumor spheroids, incubation with interleukin 2-activated lymphocytes (LAK cells) and CL158 led to structural and widespread necrosis.

L56 ANSWER 21 OF 68 CANCERLIT
AN 93687708 CANCERLIT
TI Retargeting murine T cells to react against murine breast cancer cells (Meeting abstract).
AU Moreno B; Titus J; Winkler D; Segal D; Wunderlich J
CS NIH, Bethesda, MD 20892
SO FASEB J, (1992). Vol. 6, No. 5, pp. A2059.
ISSN: 0892-6638.
DT (MEETING ABSTRACT)
FS ICDB
LA English
EM 9302
AB Previous studies have established that cytotoxic T lymphocytes, irrespective of their native specificity, can be retargeted in an MHC-independent fashion to react with other target-cell **antigens**. We have investigated here whether murine splenocytes from normal donors can be retargeted with **bispecific antibodies** to react against mouse tumor cells induced by the mammary tumor virus (MTV). The **bispecific antibody** we used reacted with CD3 triggering molecules on T cells and with gp52 **antigens** expressed by the tumor cells. C57BL/6 splenocytes were depleted of NK cells and activated in vitro with recombinant IL-2. The activated cells were then tested in the presence of **bispecific antibody** for cytolytic activity against 51Cr-labeled target cells in 4-hr assays. Antibody activity dependent on **FcR**

binding was blocked by including an anti-**FcR** antibody in the incubation. We found that T lymphocytes, retargeted with the **bispecific antibody**, reacted not only with MTV-induced tumor lines but also with freshly explanted primary tumors induced by MTV. Retargeted T cells were not cytotoxic for tumors not induced by MTV, for freshly explanted splenocytes, or for LPS-induced lymphoblasts. We conclude that murine T lymphocytes can be retargeted with **bispecific antibodies** to react selectively against murine breast cancer cells induced by MTV.

L56 ANSWER 22 OF 68 CANCERLIT
AN 92684588 CANCERLIT
TI EFFECTIVE TARGETING OF HUMAN LYMPHOCYTES AND MACROPHAGES TO TUMOR BY A BISPECIFIC MONOCLONAL ANTIBODY (BSMAB) BINDING HUMAN C-ERBB-2 AND FC GAMMA RIII (MEETING ABSTRACT).
AU Lee G; Weiner L; Ring D; Holmes M; Alpaugh K; Garcia de Palazzo I
CS Fox Chase Cancer Center, Philadelphia, PA 19111
SO Proc Annu Meet Am Assoc Cancer Res, (1992). Vol. 33, pp. A2054. ISSN: 0197-016X.
DT (MEETING ABSTRACT)
FS ICDB
LA English
EM 9208
AB BsMAbs that bind to tumor and effector cells can trigger cytotoxicity even when effector-cell **Fc** gamma receptors (**Fc** gamma R) are occupied by competing immunoglobulin. The BsMAb 2B1 is produced by a **quadroma** derived by fusion of hybridomas 520C9 (anti-c-erbB-2) and 3G8 (anti-**Fc** gamma RIII). Highly purified 2B1 is a potent promoter of in vitro lysis of c-erbB-2-expressing tumors by human large granular lymphocytes and macrophages expressing **Fc** gamma RIII. Prior cellular activation by cytokines is not required for substantial lysis, which is unimpeded in human serum or whole blood. 2B1 recognizes nearly all tumors known to overexpress c-erbB-2 protein despite its monovalent **antigen**-binding properties. Treatment of scid mice, bearing SK-OV-3 human tumor xenografts with 2B1, results in significant prolongation of survival and some cures. 2B1 meets the criteria for clinical development to test the hypothesis that BsMAbs can promote in vivo accumulation of cytotoxic effectors in tumor.

L56 ANSWER 23 OF 68 BIOSIS COPYRIGHT 1996 BIOSIS DUPLICATE 11
AN 93:24886 BIOSIS
DN BA95:13086
TI REDIRECTED TARGETING OF LDL TO HUMAN MONOCYTE FC-GAMMA RECEPTORS WITH BISPECIFIC ANTIBODIES.
AU MORGANELLI P M; KITZMILLER T J; HEMMER R; FANGER M W
CS VETERANS ADM. HOSP., RES. SERVICE 151, WHITE RIVER JUNCTION, VT 05009.
SO ARTERIOSCLER THROMB 12 (10). 1992. 1131-1138. CODEN: ARTTE5 ISSN: 1049-8834
LA English
AB Recent studies indicate that low density lipoprotein (LDL)-immune complexes consisting of anti-LDL antibodies bound to LDL may

contribute to macrophage foam cell development by uptake through immunoglobulin G (IgG) **Fc** receptors. As human mononuclear phagocytes possess three structurally and functionally distinct classes of IgG **Fc** receptors, we developed a system whereby the effects of LDL-immune complexes could be studied with respect to each type of IgG **Fc** receptor. Novel **bispecific antibodies** consisting of anti-**Fc**.gamma. receptor antibodies linked to anti-LDL antibodies were used to prepare bispecific LDL-immune complexes for targeting to specific **Fc** .gamma. receptors. In this report, the effects of bispecific LDL-immune complexes directed to **Fc**.gamma. receptor types I, II, and III were studied primarily with monocytes and were compared with the effects of similarly prepared bispecific complexes that targeted LDL to human leukocyte **antigen** (HLA) class I **antigens**. Each type of **bispecific antibody** was effective in targeting 125I-LDL to its respective site on the cell surface. Using fluorophore-labeled LDL and flow cytometry, bispecific complexes directed to **Fc**.gamma. receptor types I or II but not to HLA class I **antigens** caused a two- to sevenfold increase in cell-associated fluorescence relative to control cells treated with LDL in the absence of **bispecific antibody**. Uptake occurred in the presence of excess unlabeled LDL, acetylated LDL, and antioxidants. That the bispecific complexes triggered metabolic uptake was supported by studies of kinetics and temperature dependence. Using 125I-labeled complexes, metabolic degradation of LDL was demonstrated in association with each of the three types of **Fc**.gamma. receptors. In summary, bispecific LDL-immune complexes directed to **Fc**.gamma. receptors were taken up by monocytes, whereas similar bispecific complexes directed to HLA I **antigens** were not. Native LDL receptors and scavenger receptors were not involved. Uptake through each of the different **Fc**.gamma. receptors was associated with metabolic degradation of LDL. Bispecific reagents should be extremely useful for the analysis of pathways of lipoprotein metabolism associated with each type of **Fc**.gamma. receptor and/or other cellular structures.

L56 ANSWER 24 OF 68 BIOSIS COPYRIGHT 1996 BIOSIS DUPLICATE 12
AN 92:434689 BIOSIS
DN BA94:86814
TI BISPECIFIC MONOCLONAL ANTIBODY REGULATION OF FC-GAMMA-RIII-DIRECTED TUMOR CYTOTOXICITY BY LARGE GRANULAR LYMPHOCYTES.
AU GARCIA DE PALAZZO I; KITSON J; GERCEL-TAYLOR C; ADAMS S; WEINER L M
CS DEP. MED. ONCOL., FOX CHASE CANCER CENTER, 7701 BURHOLME AVE., PHILADELPHIA, PA. 19111.
SO CELL IMMUNOL 142 (2). 1992. 338-347. CODEN: CLIMB8 ISSN: 0008-8749
LA English
AB Bispecific monoclonal antibodies (BsMAbs) prepared by somatic cell fusion bind monovalently to their targets and yet are extremely potent enhancers of target cell lysis by relevant effector cells. The mechanisms underlying this efficiency are not known. To investigate this property, we studied the ability of selected antibodies to modulate potentiation of tumor lysis by a **bispecific**

antibody (CL158) which targets **FC** .gamma.RIII-expressing cells, via the 3G8 epitope, to malignant cells expressing CA19-9 **antigen**. Antibodies directed against the 3G8 and B73.1 epitopes of **Fc**.gamma.RII efficiently inhibited BsMAb-mediated SW948 tumor cell lysis by interleukin-2 (IL-2)-activated lymphocytes (PBLs). Unexpectedly, Leu 19 antibody reversed antibody-dependent but not antibody-independent lysis of 51Cr-labeled SW948 cells by IL-2-active PBLs in a concentration-dependent fashion. Leu 19 binds to CD56, a neural cell adhesion molecule (N-CAM) isoform expressed by large granular lymphocytes (LGLs). The effects of Leu 19 on **bispecific antibody** promotion of lysis were due to competition for binding to the 3G8 epitope of **Fc**.gamma.RIII and led to inhibition of binding between LGLs and SW948 cells. Leu 19 did not not inhibit antibody-dependent lysis by the monospecific, bivalent IgG2a variant of CA 19-9 antibody. These studies show that competition assays can be useful in dissecting the relevant mechanisms underlying BsMAb-promoted lysis. Steric constraints between effector cell trigger molecules (i.e., **Fc** .gamma.RIII) and CAM such as N-CAM may regulate the function of these molecules. Understanding the roles of diverse CAM in this phenomenon will facilitate efforts to expand and use defined effector cell populations with maximal lytic potential and to identify potentially responsive tumor phenotypes.

L56 ANSWER 25 OF 68 BIOSIS COPYRIGHT 1996 BIOSIS DUPLICATE 13
AN 92:455232 BIOSIS
DN BA94:96632
TI BISPECIFIC ANTI-HUMAN RED BLOOD RHESUS-D ANTIGEN X ANTI-FC-GAMMA-RI TARGETED ANTIBODY-DEPENDENT CELL-MEDIATED CYTOTOXICITY AND PHAGOCYTOSIS BY MONONUCLEAR LEUCOCYTES.
AU DERAMOUDT F-X; GILARD C; LEPINE N; ALONSO J-M; ROMET-LEMONNE J-L
CS FOUNDATION NATIONALE DE TRANSFUSION SANGUINE, 3 AVENUE DES TROPIQUES, B.P. 100, 91943 LES ULIS CEDEX, FRANCE.
SO CLIN EXP IMMUNOL 89 (2). 1992. 310-314. CODEN: CEXIAL ISSN: 0009-9104
LA English
AB The **Fc** receptor mediated antibody-dependent cell-mediated cytotoxicity (ADCC) and phagocytosis induced by **bispecific antibody** (BsAb) to the high-affinity **Fc** receptor for IgG (**Fc**.gamma.RI) and to human red blood group **antigen** RhD were studied in vitro, using human mononuclear leucocytes and effector cells. The results were compared by using a human monclonal IgGI anti-RhD used along and a reference human polyclonal anti-RhD antibody. The effect of non-specific human IgG on **FcR**-mediated functions by mononuclear leucocytes was checked. The results demonstrate that BsAb presents a high resistance of **Fc**-mediated function to blockade by non-specific human IgG compared with that of both polyclonal and monoclonal anti-RdD antibodies. These results further compared with that of both polyclonal and monoclonal anti-RhD antibodies. These results further encourage possible clinical application of bispecfic antibody in passive immunotherapy.

L56 ANSWER 26 OF 68 BIOSIS COPYRIGHT 1996 BIOSIS DUPLICATE 14
AN 92:211817 BIOSIS
DN BA93:112042
TI A BISPECIFIC ANTIBODY DETECTS CYTOTOXIC T LYMPHOCYTES OF UNKNOWN ANTIGEN SPECIFICITY IN PATIENTS WITH GRANULAR LYMPHOCYTE-PROLIFERATIVE DISORDERS.
AU KANEKO T; OSHIMI K; SETO T; OKUMURA K; MIZOGUCHI H
CS DEP. MED. DIV. HAEMATOL., TOKYO WOMEN'S MED. COLL., 8-1 KAWADA-CHO, SHINJUKU-KU, TOKYO 162, JAPAN.
SO BR J HAEMATOL 80 (2). 1992. 151-156. CODEN: BJHEAL ISSN: 0007-1048
LA English
AB A simple and sensitive method for the detection of cytotoxic T lymphocytes (CTL) of unknown **antigen** specificity was investigated. Using anti-CD3 Fab' .times. anti-CD10Fab' **bispecific antibody** or intact anti-CD3 monoclonal antibody, we induced cytotoxicity for CD10+. **Fc**.gamma. receptor-positive Daudi target cells in peripheral blood mononuclear cells (PBMC) obtained from patients with granular lymphocyte-proliferative disorders (GLPD). The results indicated that the **bispecific antibody** was much more efficient than the intact anti-CD3 monoclonal antibody in inducing cytotoxicity. Since CD3+CD4-CD8+ granular lymphocytes in patients with GLPD are considered to be in vivo-primed CTL of unknown **antigen** specificity, this **bispecific antibody** method may be useful for detecting in vivo-primed CTL within PBMC. By using this **bispecific antibody**, it will be possible to detect circulating in vivo-primed CTL in other clinical conditions.

L56 ANSWER 27 OF 68 MEDLINE DUPLICATE 15
AN 93119462 MEDLINE
TI Bispecific antibodies.
AU Fanger M W; Morganelli P M; Guyre P M
CS Department of Microbiology, Dartmouth Medical School, Lebanon, NH 03756..
SO CRITICAL REVIEWS IN IMMUNOLOGY, (1992) 12 (3-4) 101-24. Ref: 154
Journal code: AF1. ISSN: 1040-8401.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, ACADEMIC)
LA English
FS Priority Journals
EM 9304
AB **Bispecific antibodies**--molecules combining two different **antigenic** specificities--are currently being developed as new agents for immunotherapy and for basic studies in cell biology. **Bispecific antibodies** (BsAb) are prepared by chemically linking two different monoclonal antibodies or by fusing two hybridoma cell lines to produce a hybrid-hybridoma. Both of these approaches present challenges with respect to yield and purity that should eventually be solved through newer molecular

genetic approaches. BsAb have been used to demonstrate that specific surface molecules can trigger leukocytes to either phagocytose or kill tumor cells, viruses, parasites, and infected cells. Such trigger molecules include CD3 on T lymphocytes and **Fc** receptors for IgG on monocytes, macrophages, and natural killer cells. BsAb have also been used experimentally to localize toxins to tumor sites and fibrinolytic agents to areas of thrombosis, to study the molecular specificity of particular receptors, and as adjuvants in in vitro models of vaccines for infectious disease. The limited clinical trials that have occurred to date, primarily for therapy of tumors, suggest that BsAb may offer considerable promise for therapeutic applications, including cancer, heart disease, infectious disease, allergy, and autoimmunity.

L56 ANSWER 28 OF 68 MEDLINE DUPLICATE 16
AN 92229085 MEDLINE
TI Adoptive immunotherapy with bispecific antibodies: targeting through macrophages.
AU Chokri M; Girard A; Borrelly M C; Oleron C; Romet-Lemonne J L; Bartholeyns J
CS Centre National de Transfusion Sanguine, Les Ulis, France..
SO RESEARCH IN IMMUNOLOGY, (1992 Jan) 143 (1) 95-9.
Journal code: R6E. ISSN: 0923-2494.
CY France
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 9207
AB We report on two applications of **bispecific antibodies** to enhance the antitumoral function of human macrophages: (1) use of rhuIFN gamma (recombinant human IFN gamma) encapsulated in human red blood cells coated with anti-**Fc** gamma RI/anti-RhD+ **bispecific antibodies** to target and to activate human macrophages; encapsulated rhuIFN gamma was more potent than free IFN gamma in activating mature macrophages in vitro, demonstrating the efficacy of this delivery system to initiate in situ activation of macrophages and also to maintain a high antitumoral efficacy of macrophages with less side effects than after systemic injection of IFN gamma; (2) targeting of activated macrophages to tumours by **bispecific antibodies** directed against macrophage **Fc** gamma RI and against human adenocarcinoma **antigen**; differentiated human macrophages became cytotoxic for human adenocarcinoma in vitro and in vivo (tumours implanted in nude mice) when activated by rhuIFN gamma; this effect was increased in the presence of **bispecific antibodies**. These two approaches were aimed at increasing the efficacy of cellular immunotherapies using activated macrophages as effector cells (macrophage-activated killer, or MAK), an adoptive therapy which we have developed. **Bispecific antibodies** could increase specific homing and activation of cytotoxic MAK effectors at tumour sites.

L56 ANSWER 29 OF 68 BIOTECHDS COPYRIGHT 1996 DERWENT INFORMATION LTD

AN 92-03219 BIOTECHDS
TI Current approaches and obstacles to immunotherapy; monoclonal antibody, immunotoxin, humanized antibody, bispecific antibody engineering and application as therapeutic agents; review
AU Myers K J; *Ron Y
LO Department of Molecular Genetics and Microbiology, University of Medicine and Dentistry of New Jersey, 675 Hoes Lane, Piscataway, NJ 08854-5635, USA.
SO Pharm.Technol.; (1992) 16, 1, 26-36
CODEN: PTECDN
DT Journal
LA English
AN 92-03219 BIOTECHDS
AB The use of antibodies in immunotherapy is reviewed with regard to: (1) unconjugated monoclonal antibodies (MAbs) e.g. for tumor therapy; (2) immunoconjugates of MAbs with radioisotopes or cytostatics for targeted cancer therapy; (3) immunotoxins comprising highly potent plant, bacterial or fungal toxins attached to MAbs, used for cancer therapy, or treatment of infectious (e.g. AIDS) or autoimmune diseases; (4) humanized antibodies e.g. human antibody genes expressed in Escherichia coli; (5) antibody **heteroconjugates** (obtained by linking antibodies or Fab fragments of 2 different specificities) and **bispecific antibodies** (in which 2 Fab arms of the antibody bind different **antigens** but share a common **Fc** region); (6) approaches to T-lymphocyte mediated autoimmune disease; (7) treatment of T-cell mediated autoimmune disease by vaccination with T-cells specific for the autoantigen; (8) tumor vaccines comprising recombinant viruses expressing tumor **antigens**, or anti-idiotype MAbs; and (9) adoptive cellular therapy using lymphokine-active killer cells or cytokines (e.g. interleukins, colony stimulating factor, etc.). (76 ref)

L56 ANSWER 30 OF 68 BIOSIS COPYRIGHT 1996 BIOSIS DUPLICATE 17
AN 92:479032 BIOSIS
DN BA94:110407
TI LARGE-SCALE CULTURE SYSTEM OF HUMAN CD4-POSITIVE HELPER-KILLER T CELLS FOR THE APPLICATION TO ADOPTIVE TUMOUR IMMUNOTHERAPY.
AU NAKAMURA Y; TOKUDA Y; IWASAWA M; TSUKAMOTO H; KIDOKORO M; KOBAYASHI N; KATO S; MITOMI T; HABU S; NISHIMURA T
CS DEP. IMMUNOLOGY, TOKAI UNIV. SCH. MED., BOHSEIDAI, ISEHARA 259-11, JAPAN.
SO BR J CANCER 66 (1). 1992. 20-26. CODEN: BJCAAI ISSN: 0007-0920
LA English
AB A simple method for the rapid expansion of human CD4+ T cells with both helper and killer functions was established. CD4+ T cells separated from peripheral blood mononuclear cells using immunomagnetic beads were stimulated with immobilized OKT-3 monoclonal antibody (mAb) plus recombinant interleukin 2 (rIL-2) in 96 well culture plates. After 6 day-culture, the CD4+ T cells were restimulated by immobilized OKT-3 mAb for an additional 24 h, then inoculated into concentrated rotary-tissue culture bag and cultured

for further 9 days. This procedure yielded a 3000-fold increase in cell number (about 3-5 .times. 109 per bag). Most of the cells (over 96%) continued to express CD4+ **antigen** and retained their capacity to produce IL-2. The activated CD4+ T cells showed marked cytotoxicity against **Fc** receptor positive tumor cells in the presence of OKT-3 mAb. Moreover, we succeeded in a specific targeting of the expanded CD4+ helper/killer T cells to c-erbB-2 positive tumor cells by means of anti-CD3 .times. anti-c-erbB-2 **bispecific antibody**. These results suggested that our established simple system will be available for the expansion of large number of CD4+ helper/killer T cells whch may provide an efficient strategy for adoptive tumor immunotherapy.

L56 ANSWER 31 OF 68 SCISEARCH COPYRIGHT 1996 ISI (R)
AN 92:687388 SCISEARCH
GA The Genuine Article (R) Number: JY980
TI TARGETING OF LYMPHOCYTES-T OR LYMPHOCYTES-NK AGAINST TUMOR-CELLS BY BISPECIFIC MONOCLONAL-ANTIBODIES - ROLE OF DIFFERENT TRIGGERING MOLECULES
AU FERRINI S (Reprint); CAMBIAGGI A; CANTONI C; CANEVARI S; MEZZANZANICA D; COLNAGHI M I; MORETTA L
CS IST NAZL RIC CANC, VIA BENEDETTO XV 10, I-16132 GENOA, ITALY (Reprint); IST NAZL TUMORI, I-20133 MILAN, ITALY
CYA ITALY
SO INTERNATIONAL JOURNAL OF CANCER, (1992) Supp. 7, pp. 15-18. ISSN: 0020-7136.
DT Article; Journal
FS LIFE
LA ENGLISH
REC Reference Count: 32
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS
AB MAbs directed against triggering surface molecules expressed by T lymphocytes (CD3, TCR, CD2, CD28) or by NK cells (CD2, CD16) are able to induce the functional program of these cells. These MAbs represent suitable reagents to construct biMAbs directed against TAA, in order to specifically target effector lymphocytes against tumor cells. Anti-CD3/anti-EGF-R biMAbs were constructed to specifically direct T lymphocytes against EGF-R+ tumor cells. Such biMAb are able to induce cytolysis of EGF-R+ tumor cell lines (A431, IGROV, KATO-III and U-87) by cytolytic CD3+ effector lymphocytes while tumor cells having low or absent expression of EGF-R were not lysed. In addition, both cytolytic T (CD8+) cells and non-cytolytic (CD4+) IL-2-expanded lymphocytes were able to secrete lymphokines upon contact with EGF-R+ tumor cells. To target NK cells against NK resistant ovarian carcinomas, we used an anti-CD16 MAb (IgG1) together with an anti-ovarian carcinoma MAb (IgG2a), to construct biMAbs using the hybrid hybridoma technique. The hybrid IgG1/IgG2a biMAb triggered the specific lysis of relevant target cells by resting NK cells and by a subset of NK clones. In addition, some TCR gamma/delta+ clones but not TCR alpha/beta+ clones could be targeted by the biMAb.

L56 ANSWER 32 OF 68 DISSABS COPYRIGHT 1996 UMI

AN 91:6234 DISSABS Order Number: AAR9124217
TI FUNCTIONAL STUDIES OF HUMAN IGG FC RECEPTORS (ANTIBODY, PHAGOCYTE, CYTOTOXICITY)
AU ERBE, DAVID VINCENT [PH.D.]
CS DARTMOUTH COLLEGE (0059)
SO Dissertation Abstracts International, (1991) Vol. 52, No. 3B, p. 1335. Order No.: AAR9124217. 128 pages.
DT Dissertation
FS DAI
LA English
AB **Fc**γR trigger diverse functions which provide an interface between specific humoral immunity and nonspecific defenses of phagocytes. This thesis furthers understanding of **Fc**γR functions through the use of monoclonal and **bispecific antibodies** to dissect each function in a receptor specific manner, allowing determination of the involvement of each different **Fc**γR class, as well as non-**Fc**γR molecules, in these interactions. The studies presented include an evaluation of effector cell activation requirements for **Fc**γR dependent killing, which show that although only activation with IFN-γ increased **Fc** γR expression, activation with other cytokines enhanced **Fc**γR dependent killing in a receptor specific manner. The divalent cation requirements for ADCC by different effector cell populations were also determined, showing that lymphoid cells and myeloid cells kill opsonized tumor targets via distinct cytotoxic mechanisms, and indicating involvement of the leukocyte integrins in killing by myeloid cells. In addition, this thesis sought to extend analysis of **Fc**γR function through understanding the role of each different receptor class in the interactions between human effector cells and opsonized pathogens, by determining the role of **Fc**γR in killing of Toxoplasma gondii, and in antibody dependent enhancement of infection by dengue virus. The results indicate that no specific triggering via **Fc** γR is required for human myeloid cell ADCC of T. gondii, as measured in a short term isotope release assay. Antibody and **Fc**γR appear to function to enhance killing by myeloid cells as a result of mediating more efficient capture of the parasites. NK cell killing of T. gondii, in contrast, required specific triggering via **Fc**γRIII. Dengue virus infection of myelomonocytic, T cell and B cell lines was enhanced by bispecifics which targeted dengue to both **Fc**γR and non-**Fc**γR **antigens**, thus indicating that **Fc**γR do not provide a unique signalling function in this enhancement. Rather, antibody and **Fc**γR may enhance infection by merely focusing the virus close to the cell surface, thereby increasing the efficiency of virus receptor binding. Thus, **Fc**γR participate in functions which represent dynamic, complex processes involving interplay of various cell surface receptors. While some of these functions require unique signals triggered by **Fc**γR, others only require antibody-**Fc**γR binding.

L56 ANSWER 33 OF 68 BIOTECHDS COPYRIGHT 1996 DERWENT INFORMATION LTD
AN 91-10717 BIOTECHDS
TI Recombinant bispecific antibody human monoclonal antibody; hybridoma construction; gene cloning and expression in Escherichia coli, mammal myeloma cell culture
PA Immunomedics
PI WO 9107987 13 Jun 1991
AI WO 90-US6972 4 Dec 1990
PRAI US 89-446546 5 Dec 1989
DT Patent
LA English
OS WPI: 91-192976 [26]
AN 91-10717 BIOTECHDS
AB A **bispecific antibody**-agent conjugate for targeting foci of leukocyte accretion comprises a recombinant **bispecific antibody** (BAb) having an **antigen**-binding hypervariable region which binds specifically to granulocytes, and a constant region of a human Ig having an **Fc** portion with high affinity for receptors on human mononuclear leukocytes. The BAb is conjugated to at least one diagnostic or therapeutic agent. The BAb is prepared by immunizing a severe-combined immunodeficient mouse (repopulated with human lymphoid cells) with whole human granulocytes or membrane component fractions (as immunogen). The hyperimmunized cells are isolated and cultured as immortalized cells by Epstein-Barr virus infection, and then fused to human myeloma cells to produce human-human hybridomas, which are screened to select cells which produce useful quantities of an anti-granulocyte human monoclonal antibody. Isolated DNA encoding the essential **antigen**-binding region of the hypervariable domains is incorporated in DNA encoding the remainder of a human Ig, and expressed in Escherichia coli or mammalian myeloma cells. (37pp)

L56 ANSWER 34 OF 68 BIOTECHDS COPYRIGHT 1996 DERWENT INFORMATION LTD
AN 91-08883 BIOTECHDS
TI New monoclonal antibody specific for Fc-alpha receptor; useful for targeting human effector cells (e.g. macrophage) against a target cell such as a cancer cell or infectious agent, or for allergy therapy; bispecific antibody and hybridoma construction
PA Dartmouth-Coll.
PI WO 9105805 2 May 1991
AI WO 90-US6030 19 Oct 1990
PRAI US 89-424883 20 Oct 1989
DT Patent
LA English
OS WPI: 91-148700 [20]
AN 91-08883 BIOTECHDS
AB A new monoclonal antibody (MAb), My 43, is claimed which is specific for the **Fc**-alpha receptor of human IgA. Also claimed are **bispecific antibodies** or heteroantibodies containing the binding region derived from My 43 and the binding region of a target-specific antibody. My 43 is

used to target effector cells (e.g. macrophages) bearing the **Fc**-alpha receptor using the **bispecific antibodies** and heteroantibodies to e.g. tumor cells, autoantibody-producing lymphocytes or IgE-producing lymphocytes for allergy treatment. Microorganisms and soluble **antigens** may also be targeted. My 43 is produced conventionally by hybridoma technology. Human cells or lysates of human cells bearing the **Fc**-alpha receptor may be used for the immunization. The receptor is purified on a Sepharose column, eluting with a mildly acidic buffer. Targeted effector cells can be administered as a cell suspension using 100 million to 1 billion cells, to obtain localization at the target cells, and to effect target cell killing by antibody-dependent cytolysis. Administration is e.g. i.v. or directly into the tumor site. (12pp)

L56 ANSWER 35 OF 68 WPIDS COPYRIGHT 1996 DERWENT INFORMATION LTD
AN 91-148700 [20] WPIDS
DNC C91-064309
TI New monoclonal antibodies specific for Fc-alpha receptor - useful for targetting human effector cells e.g. macrophage(s), against target such as cancer cell or infectious agent.
DC B04 D16
IN FANGER, M W; SHEN, L
PA (DART-N) DARTMOUTH COLLEGE
CYC 16
PI WO 9105805 A 910502 (9120)*
RW: AT BE CH DE DK ES FR GB GR IT LU NL SE
W: CA JP
EP 496818 A1 920805 (9232) EN 12 pp
R: AT BE CH DE DK ES FR GB IT LI LU NL SE
JP 05504677 W 930722 (9334) 4 pp
ADT EP 496818 A1 EP 90-916618 901019, WO 90-US6030 901019; JP 05504677 W JP 90-515618 901019, WO 90-US6030 901019
FDT EP 496818 A1 Based on WO 9105805; JP 05504677 W Based on WO 9105805
PRAI US 89-424883 891020
AN 91-148700 [20] WPIDS
AB WO 9105805 A UPAB: 930928
A new monoclonal antibody (MAb) specific for human IgA receptor **Fc**-alpha, designated My43, is claimed.

Also claimed are bifunctional or hetero-antibodies comprising: (a) at least one **antigen**-binding region derived from a human anti-**Fc**-alpha receptor Ab; and (b) at least one **antigen**-binding region specific for a target epitope.

USE/ADVANTAGE - The MAbs are useful for targeting human effector cells (e.g., macrophages) against a target cell (e.g., a cancer cell or infectious agent). Targetted effector cells can specifically lyse target cells. They find use in therapy and diagnosis. For example, the **Fc**-alpha antibody can be used to target lipid vesicles contg. anticancer drugs for treating certain haematological cancers (e.g., acute myeloid leukaemia).

In an example, **bispecific antibodies** of My43 MAb and Fab anti-erythrocyte Abs were prepd. using the

coupling agent SPDP, and were shown to promote phagocytosis by monocytes, where as bi-specific Abs of anti-RBC x-anti-beta microglobulin did not. In comparative studies on phagocytosis, an average of 52% of monocytes ingested IgKG-coated RBCs and 32% ingested cells coated with My43 bi-specific Abs.
0/0

ABEQ EP 496818 A UPAB: 930928
A new monoclonal antibody (MAb) specific for human IgA receptor **Fc**-alpha, designated My43, is claimed.
Also claimed are bifunctional or hetero-antibodies comprising: (a) at least one **antigen**-binding region derived from a human anti-**Fc**-alpha receptor Ab; and (b) at least one **antigen**-binding region specific for a target epitope.
USE/ADVANTAGE - The MAbs are useful for targetting human effector cells (e.g. macrophages) against a target cell (e.g., a cancer cell or infectious agent). Targetted effector cells can specifically lyse target cells. They find use in therapy and diagnosis. For example, the **Fc**-alpha antibody can be used to target lipid vesicles contg. anticancer drugs for treating certain haematological cancers (e.g. acute myeloid leukaemia).
In an example, **bispecific antibodies** of My43 Mab and Fab anti-erythrocyte Abs were prepd. using the coupling where as bi-specific Abs of anti-RBC x-anti-beta microglobulin did not. In comparative studes on phagocytosis, an average of 52% of monocytes ingested IgKG-coated RBCs and 32% ingested cells coated with My43 bi-specific Abs

ABEQ JP05504677 W UPAB: 931119
A new monoclonal antibody (MAb) specific for human IgA receptor **Fc**-alph, designated My43, is claimed.
Also claimed are bifunctional or hetero-antibodies comprising: (a) at least one **antigen**-binding region derived from a human anti-**Fc**-alpha receptor Ab; and (b) at least one **antigen**-binding region specific for a target epitope.
USE/ADVANTAGE - The MAbs are useful for targeting human effector cells (e.g., macrophages) against a target cell (e.g., a cancer cell or infectious agent). Targetted effector cells can specifically lyse target cells. They find use in therapy and diagnosis. For example, the **Fc**-alpha antibody can be used to target lipid vesicles contg. anticancer drugs for treating certain haematological cancers (e.g., acute myeloid leukaemia).
In an example, **bispecific antibodies** of My43 MAb and Fab anti-erythrocyte Abs were prepd. using the coupling agent SPDP, and were shown to promote phagocytosis by monocytes, where as bi-specific Abs of anti-RBC x-anti-beta microglobulin did not. In comparative studies on phagocytosis, an average of 52% of monocytes ingested IgKG-coated RBCs and 32% ingested cells coated with My43 bi-specific Abs.

L56 ANSWER 36 OF 68 BIOSIS COPYRIGHT 1996 BIOSIS DUPLICATE 18
AN 92:29212 BIOSIS
DN BA93:18487
TI FC RECEPTORS FOR IGG FC-GAMMA-RS ON HUMAN MONOCYTES AND MACROPHAGES ARE NOT INFECTIVITY RECEPTORS FOR HUMAN IMMUNODEFICIENCY VIRUS TYPE 1

HIV-1 STUDIES USING BISPECIFIC ANTIBODIES TO TARGET HIV-1 TO VARIOUS MYELOID CELL SURFACE MOLECULES INCLUDING THE FC-GAMMA-R.

AU CONNOR R I; DINCES N B; HOWELL A L; ROMET-LEMMONE J L; PASQUALI J L; FANGER M W

CS DEP. MICROBIOL., DARTMOUTH MED. SCH., HANOVER, N.H. 03756.

SO PROC NATL ACAD SCI U S A 88 (21). 1991. 9593-9602. CODEN: PNASA6 ISSN: 0027-8424

LA English

AB **Fc**.gamma.Rs (**Fc**.gamma.RI, **Fc**.gamma.RII, and **Fc**.gamma.RIII) are highly expressed on human mononuclear phagocytes and function in the clearance of immune complexes and opsonized pathogens. We have examined the role of **Fc**.gamma.R in mediating antibody-dependent clearance of HIV-1 by human monocytes and monocyte-derived macrophages by using **bispecific antibodies** (BsAbs) to independently target the virus to **Fc**.gamma.RI, **Fc**.gamma.RII, or **Fc**.gamma.RIII. Virus production was markedly reduced in monocytes cultured with strain HIV-1IIIB opsonized with BsAbs that target the virus to either **Fc**.gamma.RI or **Fc** .gamma.RII compared to monocytes cultured with virus in the absence of BsAbs or in the presence of BsAbs that target the virus to non-**Fc**.gamma.R surface **antigens** (CD33 and HLA-A,B,C). These results were confirmed using the monotropic isolate HIV-1JRFL. Interaction of HIV-1JRFL with **Fc**.gamma.RI or **Fc** .gamma.RII on human monocytes and **Fc**.gamma.RI, **Fc** .gamma.RII, or **Fc**.gamma.RIII on monocyte-derived macrophages resulted in markedly reduced levels of virus production in these cultures. Moreover, HIV-1 infection of monocytes and monocyte-derived macrophages was completely blocked by anti-CD4 monoclonal antibodies, indicating that interaction with CD4 is required for infectivity even under conditions of antibody-mediated binding of HIV-1 to **Fc**.gamma.R. Thus, we propose that highly opsonized HIV-1 initiates high-affinity multivalent interactions with **Fc**.gamma.R that trigger endocytosis and intracellular degradation of the antibody-virus complex. At lower levels of antibody opsonization, there are too few interactions with **Fc**.gamma.R to initiate endocytosis and intracellular degradation of the antibody-virus complex, but there are enough interactions to stabilize the virus at the cell surface, allowing antibody-dependent enhancement of HIV-1 infection through high-affinity CD4 interactions. However, our results suggest that interaction of highly opsonized HIV-1 with **Fc**.gamma.Rs through BsAbs may reduce viral infectivity through **Fc** .gamma.R-mediated cytotoxic mechanisms and, therefore, that BsAbs offer promise as therapeutic reagents in HIV-1 infections.

L56 ANSWER 37 OF 68 SCISEARCH COPYRIGHT 1996 ISI (R)

AN 91:641471 SCISEARCH

GA The Genuine Article (R) Number: GQ080

TI A HUMANIZED MONOVALENT CD3 ANTIBODY WHICH CAN ACTIVATE HOMOLOGOUS COMPLEMENT

AU ROUTLEDGE E G (Reprint); LLOYD I; GORMAN S D; CLARK M; WALDMANN H

CS UNIV CAMBRIDGE, DEPT PATHOL, DIV IMMUNOL, TENNIS COURT RD, CAMBRIDGE

CB2 1QP, ENGLAND (Reprint)
CYA ENGLAND
SO EUROPEAN JOURNAL OF IMMUNOLOGY, (1991) Vol. 21, No. 11, pp. 2717-2725.
DT Article; Journal
FS LIFE
LA ENGLISH
REC Reference Count: 61
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS
AB The rat monoclonal antibody (mAb) YTH12.5, specific for the CD3 antigen complex on human T cells has been modified in order to improve its efficacy in human therapy. With the aim of rendering it less immunogenic, it has been humanized using the method of framework grafting. During this process sequence analysis of the YTH12.5 V(L) gene indicated that it was of the lambda subclass, however, it was markedly dissimilar from previously published rat and mouse V-lambda gene sequences and may represent a new V-lambda gene family. The humanization of this light chain represents the first successful reshaping of a lambda light chain V region. To improve the effector function of the antibody we have created a monovalent form (1 Fab, 1 Fc) using a novel method involving the introduction of an N-terminally truncated human IgG1 heavy chain gene into cells producing the humanized CD3 mAb. Comparison of the mono- and bivalent humanized mAb in a complement-mediated cell lysis assay revealed that the monovalent antibody mediated lysis of human T cell blasts whereas the bivalent form did not. The availability of a humanized, complement-fixing CD3 mAb may improve opportunities for human therapy, in the management of organ rejection, autoimmunity and the treatment of T cell lymphoma.

L56 ANSWER 38 OF 68 BIOSIS COPYRIGHT 1996 BIOSIS DUPLICATE 19
AN 91:525161 BIOSIS
TI TARGET CELL-INDUCED T CELL ACTIVATION WITH BISPECIFIC AND TRISPECIFIC ANTIBODY FRAGMENTS.
AU JUNG G; FREIMANN U; VON MARSCHALL Z; REISFELD R A; WILMANNS W
CS MED. KLIN. III, UNIV. MUENCHEN, KLIN. GROSSHADERN, D-8000 MUENCHEN, FRG.
SO EUR J IMMUNOL 21 (10). 1991. 2431-2436. CODEN: EJIMAF ISSN: 0014-2980
LA English
AB Previouslyu we proposed a concept for tumor immunotherapy in which two different **bispecific antibody** conjugates, an anti-target .times. anti-CD3 and an anti-target .times. anti-CD28 conjugate, induce the activation of resting human T cells upon binding to the respective tumor target cells. After in vivo application of these reagents, this model of a "target cell-induced T cell activation" envisages the destruction of target cells by in situ activated T cells. Obviously however, for in vivo application, the use of **Fc**-free antibody fragments is mandatory to prevent binding of the conjugates to **Fc** receptor-positive cells which would lead to **Fc**-mediated T cell activation. Here we report a similification of published procedures for the generation of bispecific Fab-hybrid fragments, univalent for each **antigen**

. We demonstrate that an anti-target .times. anti-CD3/anti-target .times. anti-CD28 combination of such hybrids, as well as an identical combination of covalently coupled F(ab')2 fragments, mediate "target cell-induced T cell activation" in an in vitro test system. Thus, these reagents may be capable of inducing an in situ activation of human T cells upon systemic in vivo application according to the concept outlined above. A trispecific conjugate with anti-target, anti-CD3-and anti-CD28 specificity appears to be unsuitable for this purpose because it activates resting T cells in soluble form without requiring immobilization through binding via its anti-target portion.

L56 ANSWER 39 OF 68 CANCERLIT
AN 92679009 CANCERLIT
TI FUNCTIONAL STUDIES OF HUMAN IGG FC RECEPTORS.
AU Erbe D V
CS Dartmouth Coll.
SO Diss Abstr Int [B], (1991). Vol. 52, No. 3, pp. 1335.
ISSN: 0419-4217.
DT (THESIS)
FS ICDB
LA English
EM 9204
AB **FcgammaR** trigger diverse functions which provide an interface between specific humoral immunity and nonspecific defenses of phagocytes. This thesis furthers understanding of **FcgammaR** functions through the use of monoclonal and **bispecific antibodies** to dissect each function in a receptor-specific manner, allowing determination of the involvement of each different **FcgammaR** class, as well as non-**FcgammaR** molecules, in these interactions. The studies presented include an evaluation of effector cell activation requirements for **FcgammaR**-dependent killing, which show that although only activation with IFN-gamma increased **FcgammaR** expression, activation with other cytokines enhanced **FcgammaR**-dependent killing in a receptor-specific manner. The divalent cation requirements for ADCC by different effector cell populations were also determined, showing that lymphoid cells and myeloid cells kill opsonized tumor targets via distinct cytotoxic mechanisms, and indicating involvement of the leukocyte integrins in killing by myeloid cells. In addition, this thesis sought to extend analysis of **FcgammaR** function through understanding the role of each different receptor class in the interactions between human effector cells and opsonized pathogens, by determining the role of **FcgammaR** in killing of Toxoplasma gondii, and in antibody-dependent enhancement of infection by dengue virus. The results indicate that no specific triggering via **FcgammaR** is required for human myeloid cell ADCC of T. gondii, as measured in a short term isotope release assay. Antibody and **FcgammaR** appear to function to enhance killing by myeloid cells as a result of mediating more efficient capture of the parasites. NK cell killing of T. gondii, in contrast, required specific triggering via **FcgammaRIII**. Dengue virus

infection of myelomonocytic, T cell and B cell lines was enhanced by bispecifics which targeted dengue to both **FcgammaR** and non-**FcgammaR antigens**, thus indicating that **FcgammaR** do not provide a unique signalling function in this enhancement. Rather, antibody and **FcgammaR** may enhance infection by merely focusing the virus close to the cell surface, thereby increasing the efficiency of virus receptor binding. Thus, **FcgammaR** participate in functions which represent dynamic, complex processes involving interplay of various cell surface receptors. While some of these functions require unique signals triggered by **FcgammaR**, others only require antibody-**FcgammaR** binding. (Full text available from University Microfilms International, Ann Arbor, MI, as Order No. AAD91-24217).

L56 ANSWER 40 OF 68 MEDLINE DUPLICATE 20
AN 92092455 MEDLINE
TI Bi-specific F(ab')2 enhances cytolytic activities of LAK cells against human small cell lung cancer (SCLC).
AU Azuma A; Niitani H
CS Department of Pulmonary Disease, Nippon Medical School..
SO NIPPON KYOBU SHIKKAN GAKKAI ZASSHI. JAPANESE JOURNAL OF THORACIC DISEASES, (1991 Sep) 29 (9) 1132-7.
Journal code: KQD. ISSN: 0301-1542.
CY Japan
DT Journal; Article; (JOURNAL ARTICLE)
LA Japanese
EM 9204
AB In order to enhance cytolytic activity of lymphokine activated killer (LAK) cells against human small cell lung cancer (SCLC), bispecific F(ab')2 was used with human LAK cells and human SCLC cell lines (i.e. N231 and H69) as targets. For the construction of bispecific F(ab')2, LU246 monoclonal antibody (mAb) recognizing the **antigen** expressed on the surface of both SCLC cell lines, was employed. OKT3 or 3G8 mAbs recognizing CD3 on T cell and **CD16** on NK cell functional molecules respectively, were chemically cross-linked to LU246 mAb using 5,5'-dithio-bis 2-nitrobenzoic acid (DTNB). Bispecific F(ab')2 adding to LAK cells increased the conjugation with target cells. The phenotypes of LAK cells change at their various culture times. Although cytolytic activity of LAK cells gradually decreases after one week of culture time, the enhanced cytotoxicity of LAK cell using bispecific F(ab')2 has been kept even after long culture. Furthermore, target cell lysis enhanced by bispecific F(ab')2 has been greater than that by classical ADCC using LU246 mAb. These **bispecific antibodies** should be effective reagents for adoptive immunotherapy in human SCLC patients.

L56 ANSWER 41 OF 68 BIOSIS COPYRIGHT 1996 BIOSIS DUPLICATE 21
AN 92:167848 BIOSIS
DN BA93:90173
TI THE ENHANCEMENT OF CYTOLYTIC ACTIVITY IN LYMPHOKINE ACTIVATED KILLER CELLS USING BISPECIFIC FAB'-2.
AU AZUMA A

CS DEP. CLINICAL PATHOL., NIPPON MEDICAL SCH., 1-1-5 SENDAGI, BUNKYO-KU, TOKYO 113, JPN.
SO J NIPPON MED SCH 58 (6). 1991. 663-672. CODEN: NIDZAJ ISSN: 0048-0444
LA Japanese
AB In this study, we show that bispecific hetero-F(ab')2 enhances cytolytic activity in human lymphokine activated killer (LAK) cells derived from peripheral blood mononuclear cells against human small cell lung cancer (SCLC) cell lines. We used two types of bispecific F(ab')2 (anti-CD3-LU246mAb, anti-**CD16**-LU246mAb) which play different roles in the enhancement of cytolytic activity in LAK cells against the human SCLC cell lines N231, H69 and Lu135. Anti-CD3 Fab' or anti-**CD16** Fab' were coupled with LU246 Fab' that recognized the **antigen** on human SCLC cells for reproducting bispecific F(ab')2. Anti-**CD16** (3G8) Fab' conjugated with LU246 Fab' targeted NK-LAK cells to SCLC cells to mediate cytolysis, but NK-LAK cells induced by LGL-enriched fraction did not display enhanced cytotoxicity even when **bispecific antibody** was used in 51Cr release assay. Anti-CD3 (OKT3) Fab' conjugated with LU246 Fab' cross-linked T-LAK cells to SCLC cells activated T-LAK cells through the CD3 complex, and enhanced the cytolytic activity of T-LAK cells against SCLC lines. Although OKT3-LU246 F(ab')2 was not so potent in enhancing cytolytic activity in 51Cr release assay, it played a greater role in enhancing the inhibitory effect on tumor growth than 3G8-LU246 F(ab')2 in human tumor clonogenic assay and in vivo tumor neutralization assay. In addition, the enhancement of target cell lysis by **bispecific antibodies** was generally more potent than antibody dependent cellular cytotoxicity (ADCC) using LU246 monoclonal antibody.

L56 ANSWER 42 OF 68 MEDLINE DUPLICATE 22
AN 92067972 MEDLINE
TI Retrovirus-derived shuttle vectors in the generation of murine bispecific MoAbs to be used in ex vivo and in vivo immunotherapy.
AU Demonte L B; Dellabona P; Nistic`o P; Tecce R; Camagna M; Reyna A; Momo M; Natali P G; Mariani M; Malavasi F
CS Dipartimento di Genetica, Biologia e Chimica Medica, Universit`a degli Studi, Turin, Italy..
SO ANNALI DELL ISTITUTO SUPERIORE DI SANITA, (1991) 27 (1) 133-7. Journal code: 5BP. ISSN: 0021-2571.
CY Italy
DT Journal; Article; (JOURNAL ARTICLE)
LA English
EM 9203
AB Mouse monoclonal antibodies specific for CD3, **FcR** and a melanoma associated **antigen** have been produced. Drug resistence of such hibrydomas has been obtained tranfecting them with plasmids containing genes conferring specific resistence. Retrovirus derived shuttle vectors with high transfection efficiency have been used for transfection. Hybrydomas were than fused and **bispecific antibody** producing cells selected.
Purification was performed by HPLC. Such **bispecific antibodies** can be used in ex vivo and in vivo immunotherapy.

L56 ANSWER 43 OF 68 MEDLINE DUPLICATE 23
AN 92067994 MEDLINE
TI Induction of functional activities of human lymphocytes by monoclonal antibodies.
AU Melioli G; Prigione I; Merl`i A; Cantoni C; Chen Q; Machi A M; Ferrini S
CS Istituto Nazionale per la Ricerca sul Cancro, Genoa, Italy..
SO ANNALI DELL ISTITUTO SUPERIORE DI SANITA, (1991) 27 (1) 79-85. Ref: 27
Journal code: 5BP. ISSN: 0021-2571.
CY Italy
DT Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
LA English
EM 9203
AB Monoclonal antibodies (MoAbs) specific for surface molecules involved in lymphocyte activation, represent an useful tool for the analysis of the activation pathways of different effector lymphocytes. We have analyzed the ability of MoAbs directed against "triggering" surface molecules, such as CD3/TCR, CD2 and **CD16** to induce activation of the lytic machinery in T and NK lymphocytes, using a redirected killing assay. In addition, we have constructed bispecific monoclonal antibodies (BiMoAbs) directed against both the CD3/TCR complex (or the **CD16** molecule) and a tumor associated **antigen** expressed on the surface of ovarian cancer cells. BiMoAbs were constructed by two different approaches: a) conjugation of two distinct MoAbs by a chemical **heterobifunctional** agent; b) selection of hybrid-hybridomas secreting BiMoAbs. BiMoAbs were able to focus appropriate lymphoid effector cells on tumor cells expressing the relevant **antigen**, and to induce tumor cell lysis. Therefore, these reagents were able to confer a new specificity for a given **antigen** to effector lymphocytes, independently from the original one. For these properties, BiMoAbs may represent a suitable tool for new strategies of adoptive immunotherapy, based on the use of effector cells that have been specifically armed by BiMoAbs against the tumor.

L56 ANSWER 44 OF 68 BIOSIS COPYRIGHT 1996 BIOSIS DUPLICATE 24
AN 91:295936 BIOSIS
TI INTERLEUKIN-2 ACTIVATED T CELLS T-LAK EXPRESS **CD16** **ANTIGEN** AND ARE TRIGGERED TO TARGET CELL LYSIS BY **BISPECIFIC ANTIBODY.**
AU NITTA T; NAKATA M; YAGITA H; OKUMURA K
CS DEP. NEUROL. NEUROLOGICAL SCI., STANFORD UNIV. SCH. MED., STANDFORD CALIF. 94305-5235, USA.
SO IMMUNOL LETT 28 (1). 1991. 31-38. CODEN: IMLED6 ISSN: 0165-2478
LA English
AB Human PBMCs from healthy donors were cultured with 100 U/ml rIL-2 for up to 5 weeks and tested at short and long activation times for the ability to mediate CD3 and CD16 targeted cytotoxicity using

chemically cross-linked bispecific antibodies. At each period, LAK activity was augmented with the use of bispecific antibodies (BA), whereas interestingly enough, at later periods (4-5 weeks) when CD16 positive lymphocytes are not present by flow cytometry, CD16 targeted cytotoxicity was induced. We suspected the possibiity of CD16 expression on activated T cells and have purified the T cell subpopulations to see the targeted cytotoxicity. Populations enriched for T cells by Percoll density centrifugation, treatment with anti-CD16 plus complement or sorting for CD5+ cells, were all able to mediate CD16 targeted cytotoxicity following activation with rIL-2. These data suggest that IL-2 activated T cells express CD16 in addition to CD3.

L56 ANSWER 45 OF 68 BIOTECHDS COPYRIGHT 1996 DERWENT INFORMATION LTD
AN 91-01673 BIOTECHDS
TI Monoclonal antibody specific for an immunoglobulin isotype; for determination of IgE and IgE producing cells and in allergy therapy and prophylaxis; hybridoma construction; chimeric antibody and bispecific antibody
PA CIBA-Geigy
PI EP 396505 7 Nov 1990
AI EP 90-810326 26 Apr 1990
PRAI GB 89-10263 4 May 1989
DT Patent
LA English
OS WPI: 90-336905 [45]
AN 91-01673 BIOTECHDS
AB A monoclonal antibody (MAb) directed against isotypic determinants of IgE which inhibits binding of free IgE to cells bearing **Fc**-epsilon receptors I or II and recognizes IgE expressed on the surface of IgE surface positive B lymphocytes is claimed. The MAb is preferably produced by fusion of mouse myeloma cells and B-lymphocytes of rats immunized with mouse IgE to obtain hybridoma 654-1-5 (ECACC 89041902), or between mouse myeloma cells and B-lymphocytes of a syngeneic mouse immunized with human IgE to give hybridoma F1-43-17 (ECACC 90032210). Also claimed are: a chimeric MAb consisting of mouse variable regions and human constant regions; a **bispecific antibody** comprising 2 different **antigen** binding portions, 1 derived from a MAb and the other from an antibody derived with a different specificity; a **bispecific antibody** in which the second **antigen** binding portion is derived from an antibody directed against a cytostatic or cytotoxic substance; and a MAb conjugated with an enzyme, fluorescent, luminescent or chemical marker. The MAb is useful for the determination of IgE and IgE-producing cells and in the treatment and prophylaxis of allergy. (30pp)

L56 ANSWER 46 OF 68 BIOSIS COPYRIGHT 1996 BIOSIS DUPLICATE 25
AN 91:51315 BIOSIS
DN BA91:29596
TI POTENTIATION OF TUMOR LYSIS BY A **BISPECIFIC ANTIBODY** THAT BINDS TO CA19-9 **ANTIGEN** AND THE

FC-GAMMA RECEPTOR EXPRESSED BY HUMAN LARGE GRANULAR LYMPHOCYTES.

AU GARCIA DE PALAZZO I; GERCEL-TAYLOR C; KITSON J; WEINER L M

CS DEP. MEDICAL ONCOLOGY, FOX CHASE CANCER CENTER, 7701 BURHOLME AVENUE, PHILADELPHIA, PA. 19111.

SO CANCER RES 50 (22). 1990. 7123-7128. CODEN: CNREA8 ISSN: 0008-5472

LA English

AB Murine monoclonal antibody therapy of human cancer rarely induces clinical responses. Antibody-induced cellular infiltrates rarely accumulate at sites of tumor, even in clinically responding lesions. Thus, the ability of these antibodies to promote host effector cell-mediated lysis of tumor via antibody-dependent cellular cytotoxicity (ADCC) has not been harnessed by existing treatment approaches. One potential explanation is that ADCC requires binding of antibody Fc domains to cellular Fc.gamma. receptors, and therapeutically administered murine antibodies must compete with vast excesses of human IgG for Fc.gamma. receptor occupancy. Chemically linked antibody heteroconjugates that bind selected target and effector cell structures via distinct Fab portions can mediate lysis of malignant cells in vitro in the presence of human serum. This approach addresses a potentially major obstacle to antibody therapy. Production of bispecific monoclonal antibodies with similar specificities and superior in vivo biodistribution characteristics would thus have potential clinical applications. We have prepared and purified a bispecific, monovalent monoclonal antibody and evaluated its in vitro effects. The IgG1-secreting hybridoma line 3G8 (.alpha.-human Fc.gamma.R III) was fused with the hybridoma line CA19-9, which produces an IgG1 antibody that binds to a glycoprotein shed by gastrointestinal cancers. Multiple clones with bispecific binding properties were identified. CA19-9 .times. 3G8 clonal supernatants and purified antibody, but not the parent antibodies, efficiently mediated specific in vitro lysis of cells of the SW948 line by human large granular lymphocytes (LGLs). Human serum-resistant target cell lysis augmentation at low effector:target ratios was seen using picogram amounts of antibody. In contrast, the IgG2.alpha. variant of CA19-9, which also promotes ADCC by LGLs, was unable to augment lysis of SW948 cells when effectors were preincubated with human serum. This bispecific, monovalent monoclonal antibody is an efficient promoter of the anti-tumor effects of LGLs in physiological concentrations of human serum. In vivo models that evaluate treatment efficacy and promotion of inflammatory tumor infiltrates by bispecific monoclonal antibodies are required to access the therapeutic potential of these novel constructs.

L56 ANSWER 47 OF 68 SCISEARCH COPYRIGHT 1996 ISI (R)

AN 90:624788 SCISEARCH

GA The Genuine Article (R) Number: EH006

TI POTENTIATION OF TUMOR LYSIS BY A **BISPECIFIC ANTIBODY** THAT BINDS TO CA19-9 **ANTIGEN** AND THE **FC**-GAMMA RECEPTOR EXPRESSED BY HUMAN LARGE ANTIGRANULOCYTES LYMPHOCYTES

AU DEPALAZZO I G; GERCELTAYLOR C; KITSON J; WEINER L M (Reprint)

CS FOX CHASE CANC INST, DEPT MED ONCOL, 7701 BURHOLME AVE, PHILADELPHIA, PA, 19111
CYA USA
SO CANCER RESEARCH, (1990) Vol. 50, No. 22, pp. 7123-7128.
DT Article; Journal
FS LIFE
LA ENGLISH
REC Reference Count: 38

L56 ANSWER 48 OF 68 BIOSIS COPYRIGHT 1996 BIOSIS DUPLICATE 26
AN 90:376397 BIOSIS
DN BA90:63078
TI ENHANCED ANTIGEN IMMUNOGENICITY INDUCED BY BISPECIFIC ANTIBODIES.
AU SNIDER D P; KAUBISCH A; SEGAL D M
CS EXP. IMMUNOL. BRANCH, NATL. CANCER INST., BLDG. 10, ROOM 4B17, NATL. INST. HEALTH, BETHESDA, MD. 20892.
SO J EXP MED 171 (6). 1990. 1957-1964. CODEN: JEMEAV ISSN: 0022-1007
LA English
AB The binding protein **antigens** to APC with heterocrosslinked **bispecific antibodies** (HBAs) enhances their processing and presentation to Th cells in vitro. Here we have asked whether HBAs could also increase immune responses in vivo. We immunized mice with hen egg lysozyme (HEL) in the presence or absence of HBA, and followed antibody production after the primary challenge and after a secondary boost. We found that HBAs that bind **antigen** to MHC class I or II molecules, to **Fc** .gamma.R, but not to surface IgD, enhance the immunogenicity of HEL. HBAs that bound HEL to MHC class II molecules, for examples, decreased the amount of **antigen** required to elicit a primary anti-HEL antibody response in mice by 300-fold, and the amount required to prime for a secondary response by 103- to 104-fold. In fact, HBAs were as effective as IFA in generating antibody responses. Since adjuvants cannot be used in humans, HBAs could prove useful for immunizing people, especially in cases where, due to scarcity or toxicity, minute doses of **antigen** must be used.

L56 ANSWER 49 OF 68 BIOSIS COPYRIGHT 1996 BIOSIS DUPLICATE 27
AN 91:70897 BIOSIS
DN BA91:39557
TI VARIABLE LINKING REGION IMMUNOGENICITY USING MALARIAL PEPTIDE CARRIER PROTEIN CONJUGATES OF DEFINED COMPOSITION.
AU JONES G L; SPENCER L; MOLLARD R; SAUL A
CS QUEENSLAND INST. MED. RES., BRAMSTON TERRACE, HERSTON, QLD. 4006, AUSTRALIA.
SO IMMUNOL LETT 26 (3). 1990. 285-290. CODEN: IMLED6 ISSN: 0165-2478
LA English
AB Thirty-five overlapping peptides from both conserved and variable parts of the N-terminal region of a malarial merozoite surface **antigen** (MSA2) were synthesised using solid phase chemistry. All peptides were synthesised with an added N-terminal cysteine and purified by reverse-phase HPLC to facilitate coupling to a carrier protein diphtheria toxoid (DT) using the **hetero-**

bifunctional reagent maleimidocaproloxysuccinimide (MCS). Mice were immunised with these peptide-DT conjugates using Freund's complete adjuvant (**FCA**). The immune response of these mice was tested against peptide hapten, carrier protein (DT) and the linking region itself, using enzyme-linked immunoassay (ELISA). Although there was great variation in the immune response to each part of the immunogen construct, no significant correlation could be seen between each set of responses.

L56 ANSWER 50 OF 68 BIOTECHDS COPYRIGHT 1996 DERWENT INFORMATION LTD
AN 91-05029 BIOTECHDS
TI Formation of chimeric antibody conjugates with heterobifunctional cross-linking reagents;
chimeric antibody conjugate for diagnosis and prophylaxis; human Caski cell-specific mouse monoclonal antibody F(ab')2 fragment and human Fc fragment derivatization, mixing (conference paper)
AU Tung E; Yeh M Y; Wang S M; Yu M H; Roffler S R; Hong C C
LO National Laboratory Animal Center, National Science Council, Taipei 10636, Taiwan, Republic of China.
SO ICSU Short Rep.; (1990) 10, 123
DT Journal
LA English
AN 91-05029 BIOTECHDS
AB Chimeric antibodies were prepared by conjugating mouse F(ab')2 fragment and human **Fc** fragment using **heterobifunctional** crosslinking agents. Mouse IgG3 monoclonal antibody 1H10 specific for a surface **antigen** of Caski (human cervix carcinoma) cells was used for F(ab')2 production by pepsin (EC-3.4.23.1) treatment. Human myeloma IgG3 protein (MAX) was used for **Fc** fragment production by papain (EC-3.4.22.2) digestion. N-succinimidyl-3-(2-pyridyldithio) propionate and Traut's reagent (2-iminothiolane-HCl, 2IT) were used to derivatize the antibody fragments, which were then mixed in various combinations and dialyzed overnight. Conjugates with mol.wt. 160,000, 220,000, 320,000 and 160,000 were identified by SDS-PAGE under non-reducing conditions. The major conjugate, 160 kDa, comprised 1 mouse F(ab')2 and 1 human **Fc** fragment. The 220 kDa conjugate was a dimer of the mouse F(ab')2, while the 320 kDa conjugate was a dimer of the 160 kDa conjugate. The yield of chimeric antibody was 10-15%, and they retained 60-80% of the **antigen** binding activity of the intact 1H10 MAb and fixed complement as effectively as intact MAX or 1H10. (9 ref)

L56 ANSWER 51 OF 68 BIOSIS COPYRIGHT 1996 BIOSIS
AN 90:230606 BIOSIS
DN BR38:108744
TI HYBRID HYBRIDOMA PRODUCING **BISPECIFIC ANTIBODIES** TO HUMAN BREAST CANCER ASSOCIATED **ANTIGENS** AND HUMAN **FC** RECEPTOR III.
AU SHI T; RING D B; EATON A M; HSIEH-MA S T; KASSEL J A
CS DEP. IMMUNOL., CUTS CORP., EMERYVILLE, CALIF.
SO XIVTH INTERNATIONAL MEETING OF THE SOCIETY FOR ANALYTICAL CYTOLOGY, ASHEVILLE, NORTH CAROLINA, USA, MARCH 18-23, 1990. CYTOMETRY 0

(SUPPL. 4). 1990. 72. CODEN: CYTODQ ISSN: 0196-4763
DT Conference
LA English

L56 ANSWER 52 OF 68 CANCERLIT
AN 91662231 CANCERLIT
TI IMMUNE SYSTEM AND CANCER.
AU Anonymous
CS No affiliation given
SO Non-serial, (1989). Immune System and Cancer. Tokyo, 1988. Hamaoka T et al, eds. Philadelphia, Taylor and Francis, 347 p., 1989.
DT Book; (MONOGRAPH)
FS ICDB
LA English
EM 9103
AB This book contains the proceedings of the 19th International Symposium of The Princess Takamatsu Cancer Research Fund, which presented results in a number of areas of immune cell biology directed toward the problems of cancer. Keynote lectures addressed immunologic aspects of B-cell-derived tumors in humans and rodents and interactions among receptors in regulation of T-lymphocyte responses. These subjects were discussed: transformation by viral or cellular oncogene, growth factor and growth control, T-cell development and repertoire selection, mechanisms of **antigen** recognition, role of receptors on T cells in immune responses, tumor **antigens** and antitumor immune responses, and molecular and cellular mechanisms of antitumor immune responses. Papers addressed these topics: human retroviruses and herpes viruses in immune dysfunctions and neoplasia; neu oncogene product in cell transformation and normal development; immunologic abnormalities in human interleukin-2 (IL-2)/IL-2 receptor L chain transgenic mice; possible roles of ATL-derived factor in IL-2 receptor and **Fc** epsilon R2 gene activation in lymphocyte transformation; T-cell **antigen** receptor (TCR) as a complex signal-transducing molecule; T-cell repertoire selection in TCR transgenic mice; role of thymic-stroma-derived T-cell growth factor in the growth of immature thymocytes and T-cell repertoire selection; T-cell selection in the thymus; rearrangement, deletion, and translocation of the human delta T-cell receptor; analysis of CD4 and CD2 receptor function; mechanisms of immunodominance in T-cell recognition and applications to vaccine design; molecular basis of **antigen** presentation; immune recognition and effector function in subsets of CD4 T cell; immune mechanisms of tissue destruction in vivo; expression of TCR V beta 8 determinants on **antigen** -specific T-helper factor; molecular polymorphism and suppressive signal transduction of I-J as a second TCR for self; activation of immune responses to prevent malignant tumor growth by in vivo administration of anti-CD3 monoclonal antibody, analysis of melanoma **antigen** and its involvement in tumor-escape mechanisms; genes encoding T-cell-defined tum- **antigens**; induction of tumor-specific in vivo protective immunity with tumor-**antigen**-pulsed **antigen**-presenting cells; gene activation in type I IFN and IL-2 systems; requirements for T-cell

recognition and elimination of retrovirally transformed cells; cytotoxic T-cell clones against human autologous cancers; properties of target molecule of murine LAK cells and clones; targeting of cytotoxic cells against tumors with heterocross-linked **bispecific antibodies**; and immunotoxins for therapy of cancer, AIDS, and immune dysfunctions.

L56 ANSWER 53 OF 68 LIFESCI COPYRIGHT 1996 CSA
AN 89:106118 LIFESCI
TI Meeting report. Monoclonal antibodies for therapy, prevention and in vivo diagnosis of human disease.
SO J. BIOL. STAND., (1989) vol. 17, no. 4, pp. 381-384.
Meeting Info.: International Association of Biological Standardization Symposium. 17-19 May 1989.
DT Journal
TC Conference
FS F; W
LA English
SL English
AB To generate monoclonal antibodies (MAbs) that are more sophisticated, effective and potent, requires several novel approaches. Some of these are entirely new (rDNA) whilst others are modified versions of conventional hybridoma techniques. By selecting the right hetero-myeloma, human hybridomas can be generated with good stability and production rate over a longer period of time. Limitations will depend on the source of lymphocytes. A modified method for making bifunctional MAbs using retroviral vectors was used to confer different marker resistances as a means of selection. **Bispecific antibodies** to CD3 and **CD16 antigens** were produced. MAbs to EGF/R, C-erS B sub(2) and myc were produced using synthetic peptides. Suitable screening techniques can yield MAbs that would recognize multiple or single epitopes. Similarly to bifunctional MAbs hetero-crosslinked MAbs can be made by chemical crosslinking. This approach allows the bridging of cytotoxic cells to the target cells. At the end of the session, the meeting "Idiotype networks in biology and medicine" held last April was reviewed.

L56 ANSWER 54 OF 68 BIOSIS COPYRIGHT 1996 BIOSIS DUPLICATE 28
AN 88:289108 BIOSIS
DN BA86:17375
TI HUMAN OVARIAN CARCINOMA LYSIS BY CYTOTOXIC T CELLS TARGETED BY BISPECIFIC MONOCLONAL ANTIBODIES ANALYSIS OF THE ANTIBODY COMPONENTS.
AU MEZZANZANICA D; CANEVARI S; MENARD S; PUPA S M; TAGLIABUE E; LANZAVECCHIA A; COLNAGHI M I
CS DIV. EXPERIMENTAL ONCOL. E, IST. MAZIONALE PER LO STUDIO E LA CURA DEI TUMORI, VIA G. VENEZIAN 1, 20133 MILAN, ITALY.
SO INT J CANCER 41 (4). 1988. 609-615. CODEN: IJCNAW ISSN: 0020-7136
LA English
AB In the perspective of in vivo therapeutic applications, the monoclonal antibody (MAb) MOv18 was selected for its restricted reactivity with human ovarian carcinoma. Using the pH2.8 desorption assay, we found that the **antig n** recognized by MOv18 had a

high stability on the cell membrane and poor internalization. Therefore, a therapeutic approach which does not require internalization, i.e, the re-targeting of cytotoxic T lymphocytes (CTL) by bispecific MAbs, was investigated. MOv18 and anti-CD3 MAbs were used to produce bispecific reagents, obtained either by chemical cross-linkage (**heteroconjugates**) or by somatic hybridization techniques (hybrid MAbs). The maintenance of the binding reactivity and specificity of the bispecific MAbs was analyzed by solid-phase radioimmunoassay, immunofluorescence and cross-competition tests on the relevant target cells (ovarian carcinoma cell line OVCA 432 for MOv18 and PHA-stimulated peripheral blood mononuclear cells for anti-CD3 MAbs), and on 2 irrelevant tumor cell lines. By a 51Cr-release assay the bispecific MAbs were found to efficiently promote, a picomolar concentration, cell lysis by CTL clones, but the specificity pattern was wider than that predicted by the binding studies. The F(ab')2 fragment of one hybrid MAb mediated a lysis which was just as efficient as the entire MAb on the relevant target cells and allowed specific lysis to be distinguished from **Fc**-receptor-mediated lysis. Human immunoglobulins were unable to compete with the **Fc** receptor binding of the hybrid MAbs and therefore, in the perspective of in vivo applications, **Fc** fragment removal seems to be an essential step. Analysis of the bispecific reagents indicated that hybrid MAbs are superior to the **heteroconjugate** as far as storage stability is concerned.

L56 ANSWER 55 OF 68 BIOSIS COPYRIGHT 1996 BIOSIS DUPLICATE 29
AN 88:399375 BIOSIS
DN BA86:72014
TI CROSS LINKING OF ANTI-B16 MELANOMA MONOCLONAL ANTIBODIES TO LYMPHOKINE ACTIVATED KILLER LAK CELLS POSSIBLE ROLE IN THE THERAPY OF B16 MELANOMA.
AU EISENTHAL A; ROSENBERG S A
CS SURGERY BRANCH, NATL. CANCER INST., BUILDING 10, ROOM 2B42, BETHESDA, MD. 20892.
SO CLIN EXP METASTASIS 6 (5). 1988. 387-400. CODEN: CEXMD2 ISSN: 0262-0898
LA English
AB We have cross-linked, using succinimidyl 4-(N-maleimidomethyl) cyclohexane-1-carboxylate (SMCC) as a **heterobifunctional** reagent, anti-B16 melanoma monoclonal antibody to lymphokine activated killer (LAK) cells, independent of the **Fc** receptor. The conditions of such linkage were optimized so that the cytotoxic properties of LAK cells, as measured in a 4 h chromium release assay against fresh tumor cells, were preserved. Using the techniques described here, covalent cross linking of anti-B16 antibody to LAK cells preserved the reactivity of this antibody to **antigens** on B16 melanoma cells, and preserved the cytotoxic properties of the antibody-bound LAK cells to lyse B16 tumor cells and other tumor cells in vitro. Cross-linking antibody remained active on the surface of LAK cells for as long as 24 h after the completion of binding. Treatment of established B16 melanoma pulmonary or subcutaneous (s.c.) tumors with LAK cells cross-linked to anti-B16 melanoma monoclonal antibody did not significantly alter

their therapeutic efficacy over untreated cells. The possible explanations for these in vivo observations and suggested approaches to increase the efficacy of the cross-linked LAK cells are discussed.

L56 ANSWER 56 OF 68 MEDLINE DUPLICATE 30
AN 90062028 MEDLINE
TI Targeting of cytotoxic cells against tumors with heterocrosslinked, bispecific antibodies.
AU Segal D M; Qian J H; Garrido M A; Perez P; Winkler D F; Wunderlich J R; Snider D P; Valdayo M J; Titus J A
CS Experimental Immunology Branch, National Cancer Institute, Bethesda, Maryland 20892..
SO PRINCESS TAKAMATSU SYMPOSIA, (1988) 19 323-31.
Journal code: HHI.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 9003
AB Cytotoxic cells express specific receptors on their surfaces by which they distinguish altered or foreign cells from normal autologous cells. Recently, a method has been developed by which the natural recognition system of cytotoxic cells can be artificially manipulated, giving rise to cytotoxic cells of any desired specificity, including specificity against tumor and virally infected cells. The method for retargeting cytotoxic cells employs heterocrosslinked antibodies, in which one antibody is directed against the cytotoxic cell receptor (CCR) involved in lysis, while the second antibody is directed against a target cell structure, for example a tumor or viral **antigen**. By linking the CCR directly to the target cell, the heterocrosslinked antibodies promote the formation of effector: target conjugates and signal the cytotoxic cell to deliver a lethal hit. T cells can be targeted by **heteroconjugates** containing antibodies against components of the T cell receptor complex, e.g., Ti or CD3, while several types of antibody-dependent cellular cytotoxicity (ADCC) effector cells, including K/NK cells, macrophages, and neutrophils, are targeted using **heteroconjugates** containing antibodies against **Fc** gamma receptors. In peripheral blood from normal donors at least six types of targetable activities have been identified in vitro. In Winn type tumor neutralization assays in nude mice, targeted T and K cells can prevent the establishment of subcutaneous tumor at low effector: tumor ratios. Moreover, targeted human peripheral blood T cells cause the eradication of established intraperitoneal human ovarian carcinoma in nude mouse models. Targeted cytotoxic cells therefore hold great promise as a novel form of cancer immunotherapy in humans.

L56 ANSWER 57 OF 68 BIOSIS COPYRIGHT 1996 BIOSIS DUPLICATE 31
AN 88:30122 BIOSIS
DN BA85:17847
TI HUMAN K-NATURAL KILLER CELLS TARGETED WITH HETERO-CROSS-LINKED ANTIBODIES SPECIFICALLY LYSE TUMOR CELLS IN-VITRO AND PREVENT TUMOR

GROWTH IN-VIVO.
AU TITUS J A; PEREZ P; KAUBISCH A; GARRIDO M A; SEGAL D M
CS BUILD. 10, ROOM 4B17, NIH, BETHESDA, MD. 20892.
SO J IMMUNOL 139 (9). 1987. 3153-3158. CODEN: JOIMA3 ISSN: 0022-1767
LA English
AB We have induced fresh peripheral blood K/natural killer cells to lyse a variety of target cells by coating them with anti-**Fc** .gamma. receptor (anti-**Fc**.gamma.R) (**CD16**) antibody hetero-cross-linked with anti-target cell antibody. The cytotoxic cell mediating this activity is different, as judged by depletion studies, from the CD3+, CD8+ T cell which is targeted by anti-CD3 cross-linked to anti-target cell antibody. Targeted K cell activity from some donors is enhanced by exposure to interleukin 2 but not interferon-.gamma.; other donors exhibit high amounts of this activity without stimulation. Specificity of lysis mediated by targeted K cells is dictated by the specificity of the anti-target cell antibody within the **heteroconjugate**, and bystander cells ar not lysed by targeted K cells. Hetero-cross-linked antibodies containing anti-histocompatibility leukocyte **antigen** class I instead of anti-**Fc**.gamma.R (**CD16**) do not promote lysis, suggesting that the bridging of the target cell to **Fc**.gamma.R on the K cell is required to activate the lytic process. Lysis mediated by targeted K cells is much less inhibitable by polymerized IgG than is classical antibody-dependent cellular cytotoxicity. Fresh human melanoma cells are lysed specifically by K cells 96.5 anti-melanoma antibody. In vivo, targeted K cells prevent tumor growth at low effector to target ratios in Winn-type tumor neutralization assays. Targeted K cells may therefore provide a new immunotherapeutic approach for the destruction of detrimental cells, such as tumor and virally infected cells.

L56 ANSWER 58 OF 68 DISSABS COPYRIGHT 1996 UMI
AN 86:4158 DISSABS Order Number: AAR8611290
TI MONOCLONAL ANTIBODIES AS TARGET STRUCTURES FOR T LYMPHOCYTES (ACTIVATION, CYTOTOXIC)
AU STAERZ, UWE DIETER [PH.D.]
CS UNIVERSITY OF CALIFORNIA, SAN DIEGO (0033)
SO Dissertation Abstracts International, (1986) Vol. 47, No. 3B, p. 891. Order No.: AAR8611290. 184 pages.
DT Dissertation
FS DAI
LA English
AB Two classes of monoclonal antibodies which react with the T cell **antigen** receptor heterodimer and identify various subsets of T lymphocytes have been described. First, anti-idiotypic (clonotypic) antibodies that recognize the receptor of the cytotoxic T lymphocyte (CTL) clone G4 (H-2('b) anti-H-2('d)) have been generated. These antibodies interfere specifically with the G4 effector function. They block cytolysis mediated by G4 and stimulate secretion of immune interferon. Second, the monoclonal antibody F23.1 belongs to another set of T cell receptor antibodies since it is specific for an allotypic determinant on the (beta)-chain that is

found on 25% of peripheral T lymphocytes in most common mouse strains independent of their functional phenotype. This IgG2a antibody, either in soluble form or covalently coupled to Sepharose beads, can activate resting T cells in the presence of an IL-2-containing source of lymphokines. Both classes of monoclonal antibodies were used to focus the effector function of CTL without the limitations usually imposed on T cell recognition. The (beta)-2-microglobulin-negative R1(TL('-)) lymphoma line, and non-specific targets, could be rendered susceptible to T lymphocyte-mediated lysis after antibodies reactive with the T cell receptor were covalently attached to their cell surface. The results demonstrate that these antibodies can redirect the response of CTL independently of the expression of major histocompatibility complex molecules. These findings were exploited to discriminate between different target cells on the basis of different surface structures. One approach successfully used the **Fc** receptor on the target cell as the attachment site for the T cell receptor antibody. In another approach, tumor cells were marked by **heteroconjugates** of monoclonal antibodies that attach to the target cell via one antibody binding site exposing the second binding site against the T cell receptor on the cell surface. The results presented here demonstrate that these constructs are powerful tools for targeting tumor cells for CTL-mediated lysis. Finally, H.10.1.6, a hybrid hybridoma between F23.1 and an anti-Thy-1.1 antibody was established. This hybridoma secretes bivalent 7s antibodies that can recruit a wide spectrum of F23.1('+) CTLs to act on Thy-1.1('+) target cells.

L56 ANSWER 59 OF 68 WPIDS COPYRIGHT 1996 DERWENT INFORMATION LTD
AN 86-330242 [50] WPIDS
DNN N86-246185 DNC C86-143418
TI New immunological complexes - comprising two antibodies each of two different animal species.
DC B04 D16 S03
PA (LANS-I) LANSDORP P M; (VRIE-N) STICHTING VRIENDEN
CYC 12
PI NL 8501219 A 861117 (8650)* 15 pp
EP 205198 A 861217 (8651) EN
R: AT BE CH DE FR GB IT LI LU NL SE
US 4868109 A 890919 (8947)
ADT NL 8501219 A NL 85-1219 850429; EP 205198 A EP 86-200734 860428; US 4868109 A US 86-855688 860425
PRAI NL 85-1219 850429
AN 86-330242 [50] WPIDS
AB NL 8501219 A UPAB: 930922
New immunological complexes (I) comprise two antibodies (Ab1) of one animal species conjugated with two monoclonal antibodies (Ab2) of another animal species to form a cyclic tetramer, where Ab2 are directed against the **Fc** fragment of Ab1.
USE/ADVANTAGE - When the two antibodies Ab1 are different, (I) are bifunctional and can be linked to a label and reacted with an **antigen** for immunoassay purposes. (I) are simpler to construct than the analogous **hybrid antibodies**

described in Nature, 305,537 (1983).
0/2

ABEQ US 4868109 A UPAB: 930922
An immunological bifunctional complex is claimed two different antibodies of a first animal species which have been conjugated to form a cyclic tetramer with two monoclonal antibodies of a second animal species directed against the Fc-fragment of the antibodies of the first animal species.

In prepn. of the bifunctional immunological complexes, two different antibodies of a first animal species are reacted with an equimolar amt. of antibodies of a second animal species which are directed against the Fc-fragments of the antibodies of the first animal species, and that the tetrameric complex formed is isolated.

L56 ANSWER 60 OF 68 CANCERLIT
AN 87638165 CANCERLIT
TI MONOCLONAL ANTIBODIES AS TARGET STRUCTURES FOR T LYMPHOCYTES.
AU Staerz U D
CS Univ. of California, San Diego, CA
SO Diss Abstr Int (Sci), (1986). Vol. 47, No. 3, pp. 891.
ISSN: 0419-4217.
DT (THESIS)
FS ICDB
LA English
EM 8711
AB Two classes of monoclonal antibodies which react with the T cell **antigen** receptor heterodimer and identify various subsets of T lymphocytes have been described. First, anti-idiotypic (clonotypic) antibodies that recognize the receptor of the cytotoxic T lymphocyte (CTL) clone G4 (H-2b anti-H-2d) have been generated. These antibodies interfere specifically with the G4 effector function. They block cytolysis mediated by G4 and stimulate secretion of immune interferon. Second, the monoclonal antibody F23.1 belongs to another set of T cell receptor antibodies since it is specific for an allotypic determinant on the beta-chain that is found on 25% of peripheral T lymphocytes in most common mouse strains independent of their functional phenotype. This IgG2a antibody, either in soluble form or covalently coupled to Sepharose beads, can activate resting T cells in the presence of an IL-2-containing source of lymphokines. Both classes of monoclonal antibodies were used to focus the effector function of CTL without the limitations usually imposed on T cell recognition. The beta-2-microglobulin-negative R1TL- lymphoma line, and non-specific targets, could be rendered susceptible to T lymphocyte-mediated lysis after antibodies reactive with the T cell receptor were covalently attached to their cell surface. The results demonstrate that these antibodies can redirect the response of CTL independently of the expression of major histocompatibility complex molecules. These findings were exploited to discriminate between different target cells on the basis of different surface structures. One approach successfully used the **Fc** receptor on the target cell as the attachment site for the T cell receptor antibody. In another approach, tumor cells were marked by

heteroconjugates of monoclonal antibodies that attach to the target cell via one antibody binding site exposing the second binding site against the T cell receptor on the cell surface. The results presented here demonstrate that these constructs are powerful tools for targeting tumor cells for CTL-mediated lysis. Finally, H.10.1.6, a hybrid hybridoma between F23.1 and an anti-Thy-1.1 antibody was established. This hybridoma secretes bivalent 7s antibodies that can recruit a wide spectrum of F23.1+ CTLs to act on Thy-1.1+ target cells.

L56 ANSWER 61 OF 68 BIOSIS COPYRIGHT 1996 BIOSIS DUPLICATE 32
AN 84:347276 BIOSIS
DN BA78:83756
TI PREPARATION AND SOME PROPERTIES OF DIMERIC RABBIT IMMUNO GLOBULIN G ANTIBODY.
AU MOTA G; SJOQUIST J; GHETIE V
CS DEPARTMENT OF IMMUNOLOGY, BABES INSTITUTE, 76201 BUCHAREST, ROMANIA.
SO MOL IMMUNOL 21 (7). 1984. 641-646. CODEN: MOIMD5 ISSN: 0161-5890
LA English
AB By reacting rabbit IgG with the fragment AB of protein A from Staphylococcus aureus (MW 14,000), an IgG dimer was formed with a MW of .apprx. 320,000 and a molar composition of IgG2-AB1. A hybrid dimer with **dual specificity** consisting of IgG anti-sheep red blood cells/AB/IgG anti-bovine red blood cells was also obtained by reacting successively both rabbit antibodies with the AB fragment. The immunologic properties (affinity for **antigen**, complement activation and binding to **Fc** receptors) of the dimeric IgG were investigated in comparison with monomeric rabbit IgG and a tetrameric IgG obtained by reaction with protein A, (IgG-protein A1)2.

L56 ANSWER 62 OF 68 LIFESCI COPYRIGHT 1996 CSA
AN 83:38308 LIFESCI
TI Preparation and applications of multivalent antibodies with dual specificity.
IMMUNOCHEMICAL TECHNIQUES. PART E. MONOCLONAL ANTIBODIES AND GENERAL IMMUNOASSAY METHODS.
AU Ghetie, V.; Moraru, I.; Langone, J.J. [editor]; Van Vunakis, H. [editor]
CS Dep. Immunol., Victor Babes Inst., 76201, Bucharest, Romania
SO METHODS ENZYMOL., (1983) pp. 523-543.
ISBN: 0-12-281992-2.
DT Book
FS F
LA English
AB In order to prepare multivalent **hybrid antibody** with double specificity, the authors used the combination of two antibodies with different specificity by means of protein A of Staphylococcus aureus (SpA), able to link the IgG molecules through their **Fc** region. Rabbit IgG treated with SpA forms a soluble complex with a molar ratio of two molecules of IgG per molecule of SpA and molecular composition (IgG-SpA-IgG) sub(2). Linkage of IgG antibodies with two different specificities (anti-A

and anti-B) by SpA is thought to occur at random, yielding a multivalent **hybrid antibody** complex (anti-A/SpA/anti-B) sub(2) mixed with other antibody complexes consisting of (anti-A/SpA/anti-A) sub(2) and (anti-B/SpA/anti-B) sub(2). The last two unwanted antibody complexes cannot be removed by successive reaction with the corresponding **antigens**, since acid dissociation of the **antigen**-antibody complex would also break the linkage SpA and the two IgG antibody molecules of the hybrid complex.

L56 ANSWER 63 OF 68 BIOSIS COPYRIGHT 1996 BIOSIS DUPLICATE 33
AN 81:288750 BIOSIS
DN BA72:73734
TI SIMULTANEOUS DETECTION OF 2 CELL SURFACE ANTIGENS BY ELECTRON MICROSCOPY BY MULTIVALENT HYBRID ANTIBODY WITH DOUBLE SPECIFICITY.
AU MANDACHE E; MOTA G; MOLDOVEANU E; MORARU I; GHETIE V
CS BABES INST., SPL. INDEPENDENTEI 99, R-76201 BUCHAREST, RUMANIA.
SO J IMMUNOL METHODS 42 (3). 1981. 355-366. CODEN: JIMMBG ISSN: 0022-1759
LA English
AB Multivalent **hybrid antibody** (MHA) complexes with **dual specificity** were prepared by combining 2 antibodies of different specificities, one against ferritin (Fer), the other against horseradish peroxidase (HRP), with protein A of Staphylococcus aureus (SpA). EM of mouse spleen lymphocytes and thymocytes [TC] (previously coated with mouse IgG anti-Thy-1 antibody) treated with IgG anti-Fer/SpA/IgG anti-mouse Ig complex and Fer gave better resolution and higher accuracy than previously obtained with IgG anti-HRP/SpA/IgG anti-mouse Ig complex and HRP. Surface Thy-1 alloantigen and **Fc** receptor (charged with human IgG) treated with a mixture of IgG anti-Fer/SpA/IgG anti-Thy-1 and IgG anti-HRP/SpA/IgG anti-human Fab could be simultaneously detected on the TC surface by either light or EM using Fer and HRP. The concomitant visualization of Thy-1 alloantigen (with Fer) and **FcR** (with HRP) on mouse TC clearly shows that their distribution is largely independent and that the amount of Thy-1 **antigen** is greater. Evidently EM with a mixture of MHA is a useful technique for simultaneous location of 2 **antigenic** markers on the cell surface.

L56 ANSWER 64 OF 68 MEDLINE DUPLICATE 34
AN 82153888 MEDLINE
TI Inhibition of responder cell activity in mixed leukocyte culture reactions. I. Evidence of cross-reactivity of B7-antibody with HLA-B8.
AU de Rooij-Doyer E; Bruning J W; van Rood J J
SO TISSUE ANTIGENS, (1981 Sep) 18 (3) 154-65. Journal code: VSV. ISSN: 0001-2815.
CY Denmark
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 8207

AB In a previous report we described a human allo-antiserum which showed MHC restricted **dual specific** inhibition of mixed leukocyte culture (MLC) reactions (de Rooij et al. 1980). Responder cells were preincubated with the antiserum and washed. Inhibition of MLC reactions occurred if HLA-B8 positive responder cells and HLA-B7 or B40 positive stimulator cells were used. The mechanism of inhibition by this antiserum was investigated and described in this report. The results strongly suggested that the observed inhibition was due to HLA-B7 specific antibody molecules cross-reacting with HLA-B8 **antigens**. We decided on the following mechanism: HLA-B7 specific antibody molecules bind to HLA-B8 **antigens** on responder cells at 4 degrees C. Bound antibody molecules area readily released at 37 degrees C and the bind preferentially to HLA-B7 or -B40 positive stimulator cells. Stimulator cells are then lysed by antibody dependent cellular cytotoxicity (ADCC), resulting in impaired MLC reaction. This conclusion was derived from the following observations: 1. HLA-B7 positive cells could be lysed due to antibody molecules bound to B8 positive cells: a. Cells coated with antibody molecules lysed B7 or B40 positive target cells. b. Antibody molecules released from B8 positive cells lysed B7 or B40 positive target cells in ADCC. 2. The active antibody molecules were HLA-B7 specific: a. The inhibiting antibody molecules in the antiserum could be absorbed on and eluted from HLA-B7 positive platelets (de Rooij et al. 1980). b. Antibody molecules bound to and released from HLA-B8 positive cells carried the same B7 specific inhibiting and cytotoxic activity as the original antiserum. 3. The site of recognition on HLA-B8 positive cells is the HLA-B8 **antigen**: a. F(ab')2 preparations from HLA-B8 and -Bw6 specific antisera inhibited antibody binding to B8 positive cells. b. Antibody binding was not restricted to B, T, **FcR+**, **FcR-** or non adherent cell subpopulations. 4. The antibody molecules bind to B8 positive cells with the **antigen** binding site and not with the **Fc** part of the molecule: a. Antibody binding could be blocked partially with a F(ab')2 preparation from the original antiserum but not with a **Fc** preparation. b. The **antigen** binding site of antibody molecules bound to HLA-B8 positive cells was not freely available, since the MLC inhibiting activity of sensitised B8 positive cells could not be blocked by soluble HLA-B7 **antigens**. c. The antiserum was cytotoxic for HLA-B8 positive target cells in ADCC. The observed inhibition of MLC by antibody molecules with apparent **dual specificity** is thus the consequence of a shared or a similar **antigenic** determinant of HLA-B7 and HLA-B8 **antigen** molecules.

L56 ANSWER 65 OF 68 MEDLINE DUPLICATE 35
AN 82057841 MEDLINE
TI Electron microscopic investigation of cell surface antigens using a multivalent hybrid antibody with double specificity.
AU Mandache E; Moldoveanu E
SO MORPHOLOGIE ET EMBRYOLOGIE, (1981 Apr-Jun) 27 (2) 109-15. Journal code: NJP. ISSN: 0377-5038.
CY Romania

DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 8203
AB Multivalent **hybrid antibody** (MHA) complexes with double specificity were prepared by combining two different antibodies, one specific against ferritin (Fer), the other against horseradish peroxidase using protein A from Staphylococcus aureus (SpA). Electron microscopy of mouse spleen lymphocytes and thymocytes (the latter coated with mouse anti-Thy-1 antibody) reacted with anti-HRP/SpA/anti-mIg complex and HRP showed the pattern of surface Ig receptors visualized by the specific peroxidase labelling. When IgG anti-Fer/SpA/IgG-anti-mIg complex was applied to the same cellular stuff, better resolution and higher accuracy were obtained although the distribution was similar. Surface Thy-1 alloantigen and Fe receptors (charged with hIgG) were simultaneously detected on the thymocyte surface by using in the same way a mixture of anti-Fer/SpA/anti-Thy-1 and anti-HRP/SpA/anti-Fab. The concomitant ultrastructural visualization of Thy-1 (with Fer) and **Fc** (with HRP) on mouse thymocytes clearly showed that their distribution was mainly independent and that the amount of Thy-1 **antigen** prevailed. These data show that electron microscopy with a mixture of MHA may be a useful technique for the concomitant location of two **antigenic** determinants on the cell surface.

L56 ANSWER 66 OF 68 BIOSIS COPYRIGHT 1996 BIOSIS DUPLICATE 36
AN 80:196919 BIOSIS
DN BA69:71915
TI MAJOR HISTO COMPATIBILITY COMPLEX RESTRICTED DUAL SPECIFIC INHIBITION OF MIXED LEUKOCYTE CULTURE REACTIONS BY HUMAN HLA ANTIBODY MOLECULES.
AU DE ROOIJ-DOYER E; JONKER M; BRUNING J W; VAN ROOD J J
CS DEP. IMMUNOHAEMATOL., ACAD. ZIEKENHUIS, RIJNSBURGERWEG 10, LEIDEN, NETH.
SO TISSUE ANTIGENS 15 (1). 1980. 47-59. CODEN: TSANA2 ISSN: 0001-2815
LA English
AB A human alloantiserum was found which selectively inhibits responding cells in mixed leukocyte culture reactions. Inhibition was achieved by pre-incubation of responder cells in the antiserum followed by washing. The serum showed **dual specificity** as an inhibiting agent. Inhibition was restricted to HLA-B7 or -B40 positive stimulator cells, specificities against which the antiserum also had cytotoxic activity. Inhibition was almost exclusively associated with the presence of the phenotype HLA-A1, -B8 on the responder cells. The HLA associated specificity for responder cells was unexpected since no alloantibody activity directed to responder alloantigens could be detected by conventional serological methods. The antiserum donor had not been immunized with HLA-A1, -B8 **antigens** nor with known crossreactive **antigens**. The serum donor did not carry HLA-A1, -B8 **antigens** herself. The inhibiting substance in the antiserum had physicochemical properties of Ig[immunoglobulin]G and was specifically reactive with HLA-B7 positive platelets. Pepsin digest preparations were not inhibitory.

Fc receptor positive responder cells were required for inhibition. Responder cells preincubated with the antiserum suppressed the response of cells not incubated with the antiserum. Three possible explanations of these results were discussed: specific binding of the **Fc** part of the antibody with **Fc** receptors of responder cells, specific activation of suppressor cells and crossreactivity.

L56 ANSWER 67 OF 68 EMBASE COPYRIGHT 1996 ELSEVIER SCI. B.V.
AN 79042063 EMBASE
TI Detection of H-Y antigen on human male leukocytes.
AU Goldberg E.H.; Arrington T.; Tokuda S.
CS Dept. Microbiol., Univ. New Mexico, Sch. Med., Albuquerque, N.M. 87131, United States
SO J. IMMUNOL. METHODS, (1978) 23/3-4 (203-206).
CODEN: JIMMBG
CY Netherlands
LA English
AB Using the sperm cytotoxicity test and the 'mixed hemadsorption **hybrid antibody**' (MHA.HA) assay (Wachtel, 1977), the authors have detected H-Y (male) **antigen** in several mammalian species. Because both these methods present difficult technical problems, the authors recently developed a simplified serological assay for the detection of H-Y on mouse lymphocytes (Tokuda et al., 1977). This assay is based on the observation that the protein A component of Staphylococcus aureus (SA) binds to the **Fc** portion of IgG molecules, which was adapted by Kearney et al. (1975) to measure SA rosettes. The only limitation of this procedure is the requirement for antibodies of the IgG class, which means that its efficiency depends on the concentration of IgG antibodies in the anti-H-Y serum. But antibody of this class is in fact present in mouse H-Y antiserum, and the authors report here that they can use this technique to detect H-Y **antigen** on human leukocytes, confirming the work of Wachtel et al.

L56 ANSWER 68 OF 68 EMBASE COPYRIGHT 1996 ELSEVIER SCI. B.V.
AN 76019004 EMBASE
TI Lack of requirement for conformational change in IgG antibody in forming complement fixing immune complexes.
AU Kazmar R.E.
CS Northwest. Univ., Chicago, Ill. 60611, United States
SO FED.PROC., (1975) 34/3 (no.4181).
CODEN: FEPRA7
LA English
AB Besides the need for clustering 2 or more IgG molecules, conformational changes within the **Fc** piece may be required to bind the activated first component of complement. This latter requirement was tested with pure **hybrid antibody** prepared by combining the HL halves of rabbit IgG antibody to BSA and HGG and contrasting its complement fixing ability when bound either divalently or monovalently to **antigen**. When bound divalently to sheep red blood cells (SRBC) coated with BSA and HGG, 2 **hybrid antibody** molecules formed a CI fixing

site. The number of CI fixed was within the same range as fixed by the parent antibodies. When hydrid antibody was bound monovalently to SRBC coated only with BSA, again 2 hydrid antibodies formed a CI fixing site, but only 1/5 as many sites were formed as with divalently bound **hybrid antibody**. This lesser CI fixing site formation was probably due to the 6 times fewer **hybrid antibodies** bound monovalently than divalently. Monovalently bound **hybrid antibody** had about 1/4 the whole complement fixing activity of divalently bound **hybrid antibody**. These findings suggest that distortion due to divalent binding is not an absolute requirement for complement fixation and that a complement fixing site is an intrinsic part of the native IgG antibody molecule.

FILE COVERS 1967 - 27 Mar 1996 (960327/ED) VOL 124 ISS 14

```
=> fil ca,caplus; s (bsab or bsmab or bsmoab or bab)(l)(fc? or cd16 or cd
16)
FILE 'CA' ENTERED AT 15:51:10 ON 02 APR 96
USE IS SUBJECT TO THE TERMS OF YOUR CUSTOMER AGREEMENT
COPYRIGHT (C) 1996 AMERICAN CHEMICAL SOCIETY (ACS)

FILE 'CAPLUS' ENTERED AT 15:51:10 ON 02 APR 96
USE IS SUBJECT TO THE TERMS OF YOUR CUSTOMER AGREEMENT
COPYRIGHT (C) 1996 AMERICAN CHEMICAL SOCIETY (ACS)


L59          14 FILE CA
L60          14 FILE CAPLUS

TOTAL FOR ALL FILES
L61          28 (BSAB OR BSMAB OR BSMOAB OR BAB)(L)(FC? OR CD16 OR CD 16)

=> s l61 not l21
L62           9 FILE CA
L63           9 FILE CAPLUS

TOTAL FOR ALL FILES
L64          18 L61 NOT L21

=> s ran=,1994
ENTER LOGIC EXPRESSION, QUERY NAME, OR (END):164
L65           5 FILE CA
L66           5 FILE CAPLUS

TOTAL FOR ALL FILES
L67          10 L64

=> dup rem l67; d 1-5 .beverly; fil
biosi,medl,embas,lifesci,biotechds,cancerlit,wpids,dissabs,confsci,scisearc
h
PROCESSING COMPLETED FOR L67
L68           5 DUP REM L67 (5 DUPLICATES REMOVED)


L68  ANSWER 1 OF 5  CA  COPYRIGHT 1996 ACS              DUPLICATE 1
AN   121:169915  CA
TI   Potentiation of long-term-cultured lymphokine-activated killer cell
     cytotoxicity against small-cell lung carcinoma by anti-CD3 .times.
     anti-(tumor-associated antigen) bispecific antibody
SO   Cancer Immunol. Immunother. (1994), 38(5), 294-8
     CODEN: CIIMDN; ISSN: 0340-7004
AU   Azuma, Arata; Yagita, Hideo; Okumura, Ko; Kudoh, Shoji; Niitani,
     Hisanobu
PY   1994
AB   Lymphokine-activated killer (LAK) cells exhibit a potent
     cytotoxicity to malignant cells in vitro.  However, a satisfactory
     effect has not been obtained in many clin. studies except for a few
```

cases. One of the most important reasons why cytolytic activity could not be exhibited in vivo is that LAK cells do not accumulate in the tumor tissue because of a lack of specificity. In the present study, we show the effect of a bispecific antibody (**bsAb**) on the accumulation of LAK cells around the small-cell lung carcinoma (SCLC) cell and the subsequent enhancement of LAK cell cytotoxicity against SCLC. When short-term(4 days)-cultured LAK cells were used, OKT3.times.LU246 **bsAb**, which direct CD3+ T-LAK cells to the target cell, induced a similar level of cytotoxicity to that induced by 3G8.times.LU246 **bsAb**, which directs **CD16**+ LAK cells. Long-term(21 days)-cultured LAK cells exhibited a reduced spontaneous cytotoxicity but retained high cytotoxic activity, which could be directed by OKT3.times.LU246 or 3G8.times.LU246 **bsAb**. The inhibitory effect of LAK cells on tumor cell clonogenicity in soft agar was also enhanced by both **bsAb**. These results indicate that application of the therapy with LAK cells and OKT3.times.LU246 **bsAb** to SCLC patients might be a promising new method of adoptive immunotherapy.

L68 ANSWER 2 OF 5 CA COPYRIGHT 1996 ACS DUPLICATE 2
AN 119:268466 CA
TI Clustered CD3/TCR complexes do not transduce activation signals after bispecific monoclonal antibody-triggered lysis by cytotoxic T lymphocytes via CD3
SO J. Immunol. (1993), 151(6), 2904-14
CODEN: JOIMA3; ISSN: 0022-1767
AU Blank-Voorthuis, Carla J. A. C.; Braakman, Eric; Ronteltap, Cees P. M.; Tilly, Ben C.; Sturm, Els; Warnaar, Sven O.; Bolhuis, Reinder L. H.
PY 1993
AB Bispecific mAb (**bsmAb**) directed against the CD3/TCR complex and a tumor-assocd. antigen (Ag, TAA) induces CTL-mediated lysis of TAA+ target cells. The authors have investigated whether **bsmAb**-pretargeted CTL can enter multiple lytic cycles. **BsmAb**-pretargeted CTL retained **bsmAb**-targeted lytic capacity for at least 24 h when exposed to medium without TAA+ target cells. Exposure of **bsmAb**-pretargeted CTL to TAA+ target cells resulted in a rapid loss of **bsmAb**-targeted cytotoxicity of TAA+ or **Fc**.gamma.R+ target cells, although the CTL retained surface **bsmAb**. Moreover, addn. of rabbit anti-mouse Ig to these CTL did not induce calcium mobilization. These CTL still showed Ag-specific cytotoxicity and cytolysis of anti-CD3 mAb-expressing hybridoma cells. Readdn. of **bsmAb** to CTL that had lost **bsmAb**-targeted cytotoxicity instantly restored the **bsmAb**-targeted lytic activity of the CTL. Hence, as in Ag-specific cytolysis, **bsmAb**-pretargeted CTL can enter multiple **bsmAb**-targeted cytolytic cycles. Surprisingly, exposure of **bsmAb**-pretargeted CTL to **Fc**.gamma.R+ cells did not result in loss of **bsmAb** -targeted cytolysis of TAA+ cells. Fluorescence microscopic anal. revealed that **bsmAb**-mediated interaction with TAA+ cells, but not with **Fc**.gamma.R+ cells, resulted in clustering of

bsmAb-pretargeted CD3/TCR complexes on the CTL surface. On the basis of the obsd. correlation between clustered **bsmAb** -pretargeted CD3/TCR complexes and loss of **bsmAb**-targeted cytotoxicity, the authors hypothesize that clustered CD3/TCR complexes can no longer transduce signals.

L68 ANSWER 3 OF 5 CA COPYRIGHT 1996 ACS DUPLICATE 3
AN 119:247603 CA
TI Binding and cytotoxicity characteristics of the bispecific murine monoclonal antibody 2B1
SO J. Immunol. (1993), 151(5), 2877-86
CODEN: JOIMA3; ISSN: 0022-1767
AU Weiner, Louis M.; Holmes, Michele; Richeson, Anne; Godwin, Andrew; Adams, Gregory P.; Hsieh-Ma, Sylvia T.; Ring, David B.; Alpaugh, R. Katherine
PY 1993
AB Bispecific monoclonal antibodies (**BsmAb**) with specificity for tumor Ag and effector cell trigger mols. have been shown to redirect the cytotoxicity of several peripheral blood mononuclear cell populations against relevant tumor. The **BsmAb**, 2B1, binds to the extracellular domain of the c-erbB-2 gene product of the HER2/neu proto-oncogene and to **CD16**. The binding and cytotoxic characteristics of 2B1 were studied. Maximal satn. binding of 2B1 to PBL and c-erbB-2 expressing SK-OV-3 cells occurred in the 1 .mu.g/mL concn. range. However, substantial lysis potentiation was obsd. at 1000-fold lower **BsmAb** concns. Optimal tumor lysis was obtained when the **BsmAb**, PBL, and target cells were continuously coincubated. When PBL were franked with 2B1, washed, and added to labeled targets, substantially less lysis was obsd. These results suggest that the best way to therapeutically exploit the cytotoxic attributes of 2B1 may be to obtain continuous **BsmAb** exposure to tumor. Approaches based on franking of this **BsmAb** to PBL may not be warranted.

L68 ANSWER 4 OF 5 CA COPYRIGHT 1996 ACS DUPLICATE 4
AN 117:88246 CA
TI Comparative efficiencies of bispecific F(ab'.gamma.)2 and chimeric mouse/human IgG antibodies in recruiting cellular effectors for cytotoxicity via Fc.gamma. receptors
SO Cancer Immunol. Immunother. (1992), 34(6), 361-9
CODEN: CIIMDN; ISSN: 0340-7004
AU Greenman, John; Hogg, Nancy; Nikoletti, Suzanne; Slade, Christopher; Stevenson, George; Glennie, Martin
PY 1992
AB The 3 forms of **Fc**.gamma. receptor carried by monocytes (**Fc**.gamma.RI, II) and natural killer (NK) cells (**Fc** .gamma.RIII) are all capable of mediating cell lysis. Here are compared the use of F(ab'.gamma.)2 bispecific antibodies, specifically targetting individual **Fc**.gamma.R, and chimeric IgG mouse/human antibodies which are capable of targetting all **Fc**.gamma.R, for their ability to mediate target cell destruction. The derivs. are prepd. by linking hinge sulfhydryl

residues via tandem thioether bonds, using a bismaleimide crosslinker: Fab' from an anti-**Fc**.gamma.R mAb linked to Fab' from a common anti-target mAb (**BsAb**), or Fab' from the common anti-target mouse antibody linked to human **Fc** .gamma. (FabFc or bisFabFc). All the derivs. targetting chick red blood cells gave efficient lysis, although different effector cell donors yielded differences in both the lytic levels achieved and the comparative efficiencies of derivs. In contrast, significant lysis of the guinea pig lymphoblastic leukemia, L2C, regularly resulted only via the anti-**Fc**.gamma.RIII **BsAb** and the chimeric derivs. These results suggest that the chimeric, **Fc**-contg. derivs. mediate tumor cell lysis principally through **Fc**.gamma.RIII on NK cells. This is in contrast to the situation with the chick red blood cells where the chimeric derivs. appear capable of lysing erythrocytes by utilizing either monocytes or NK cells, because significant (.apprxeq.50%) lysis occurred with effector cell populations magnetically depleted through either **Fc**.gamma.RII or **Fc**.gamma.RIII. A major difference between these 2 types of antibody derivs. was their ability to function in the presence of high concns. of normal human **Fc**.gamma.. The lysis mediated by **BsAb** reactive with **Fc**.gamma.RI or II was unaffected by the presence of human **Fc**.gamma. at 2.5 mg/mL (a concn. comparable with that yielded by IgG in plasma) whereas the **BsAb** recognizing **Fc**.gamma.RIII and all the **Fc**-contg. derivs. were completely inhibited.

L68 ANSWER 5 OF 5 CA COPYRIGHT 1996 ACS DUPLICATE 5
AN 107:196132 CA
TI Bi-specific monoclonal antibodies: selective binding and complement fixation to cells that express two different surface antigens
SO J. Immunol. (1987), 139(4), 1369-74
CODEN: JOIMA3; ISSN: 0022-1767
AU Wong, Johnson T.; Colvin, Robert B.
PY 1987
AB A new dimension of immunotherapeutic selectivity might be achieved if antibodies distinguished cells that co-express 2 different surface antigens. Bi-specific monoclonal antibodies (**BSMAB**) with 2 different antigen combining sites that share a common **Fc** region theor. might have such a potential. Two such **BSMAB** were produced by hybrid-hybridoma clones prepd. by fusion of pre-existing hybridomas and were purified by isoelec. focusing. CD3,4 (IgG2a, IgG2b) recognizes the T cell surface antigens CD3 and CD4, and CD3,8 (IgG2a, IgG2a) recognizes CD3 and CD8. These **BSMAB** promote complement-mediated lysis of target cells that bear both surface antigens 25-3125-fold more efficiently than those that express only 1 of the antigens. This selectivity results from the increased avidity of these antibodies for cells with both antigens, as reflected by the increased surface Ig concn. detected by flow cytometry. There exists a threshold surface IgD. necessary for antibody-dependent complement-mediated cytotoxicity microtiter assays for the various IgG antibodies tested in both bivalent and monovalent binding. These results support the

associative model of IgG-mediated complement fixation.

FILE 'BIOSIS' ENTERED AT 15:53:10 ON 02 APR 96
COPYRIGHT (C) 1996 BIOSIS(R)

FILE 'MEDLINE' ENTERED AT 15:53:10 ON 02 APR 96

FILE 'EMBASE' ENTERED AT 15:53:10 ON 02 APR 96
COPYRIGHT (C) 1996 Elsevier Science B.V. All rights reserved.

FILE 'LIFESCI' ENTERED AT 15:53:10 ON 02 APR 96
COPYRIGHT (C) 1996 Cambridge Scientific Abstracts (CSA)

FILE 'BIOTECHDS' ENTERED AT 15:53:10 ON 02 APR 96
COPYRIGHT (C) 1996 DERWENT INFORMATION LTD

FILE 'CANCERLIT' ENTERED AT 15:53:10 ON 02 APR 96

FILE 'WPIDS' ENTERED AT 15:53:10 ON 02 APR 96
COPYRIGHT (C) 1996 DERWENT INFORMATION LTD

FILE 'DISSABS' ENTERED AT 15:53:10 ON 02 APR 96
COPYRIGHT (C) 1996 University Microfilms, Inc.

FILE 'CONFSCI' ENTERED AT 15:53:10 ON 02 APR 96
COPYRIGHT (C) 1996 Cambridge Scientific Abstracts (CSA)

FILE 'SCISEARCH' ENTERED AT 15:53:10 ON 02 APR 96
COPYRIGHT (C) 1996 Institute for Scientific Information (ISI) (R)

=> s (bsab or bsmab or bsmoab or bab)(l)(fc? or cd16 or cd 16)
L69 17 FILE BIOSIS
L70 26 FILE MEDLINE
L71 25 FILE EMBASE
L72 9 FILE LIFESCI
L73 2 FILE BIOTECHDS
L74 25 FILE CANCERLIT
L75 0 FILE WPIDS
L76 0 FILE DISSABS
L77 0 FILE CONFSCI
L78 11 FILE SCISEARCH

TOTAL FOR ALL FILES
L79 115 (BSAB OR BSMAB OR BSMOAB OR BAB)(L)(FC? OR CD16 OR CD 16)

=> s ran=,1994
ENTER LOGIC EXPRESSION, QUERY NAME, OR (END):179
L80 14 FILE BIOSIS
L81 15 FILE MEDLINE
L82 17 FILE EMBASE
L83 7 FILE LIFESCI

```
L84           2 FILE BIOTECHDS
L85          20 FILE CANCERLIT
L86           0 FILE WPIDS
L87           0 FILE DISSABS
L88           0 FILE CONFSCI
L89           8 FILE SCISEARCH

TOTAL FOR ALL FILES
L90          83 L79

=> s l90 not l55
L91           9 FILE BIOSIS
L92           9 FILE MEDLINE
L93           9 FILE EMBASE
L94           5 FILE LIFESCI
L95           0 FILE BIOTECHDS
L96          14 FILE CANCERLIT
L97           0 FILE WPIDS
L98           0 FILE DISSABS
L99           0 FILE CONFSCI
L100          5 FILE SCISEARCH

TOTAL FOR ALL FILES
L101         51 L90 NOT L55

=> dup rem l101
PROCESSING COMPLETED FOR L101
L102          22 DUP REM L101 (29 DUPLICATES REMOVED)

=> d 1-22 bib abs; fil ca,caplus; d que 157

L102 ANSWER 1 OF 22  CANCERLIT
AN   94602997  CANCERLIT
TI   Phase Ia/Ib trial of bispecific monoclonal antibody (BsAb) therapy
     (anti-Her-2/neu x anti-CD64) (MDX-210) for breast or ovarian cancers
     that over express Her-2/neu (Meeting abstract).
AU   Valone F H; Kaufman P A; Fanger M W; Guyre P M; Memoli V
CS   Dartmouth-Hitchcock Medical Center, Lebanon, NH 03756
SO   Proc Annu Meet Am Assoc Cancer Res, (1994). Vol. 35, pp. A1311.
     ISSN: 0197-016X.
DT   (MEETING ABSTRACT)
     (CLINICAL TRIAL, PHASE I)
     (CLINICAL TRIAL)
FS   ICDB
LA   English
EM   9411
AB   MDX-210 is a Fab' x Fab' BsAb constructed by chemically
     crosslinking Fab' mAb 520C9 (anti-Her-2/neu) and Fab' mAb22
     (anti-CD64). CD64 is a high affinity Fc receptor for IgG.
     In vitro, MDX-210 effectively directs cytotoxicity and phagocytosis
     by monocyte-derived macrophages and IFNgamma-treated neutrophils.
     MDX-210 triggers release of TNFalpha by macrophages in the presence,
     but not in the absence, of HER-2/neu expressing target cells. The
```

clinical trial's endpoints are evaluation of toxicity, determination of the MTD, and biological efficacy. Patients (pts) with stage IV breast cancer or stage III or IV ovarian cancer that is resistant to standard therapy and expresses Her-2/neu are eligible for treatment. Eight pts have been treated. Dose levels tested are 0.35, 1.0 and 3.5 mg/m2 given IV once at 6 mg/hr. Treatment was well tolerated. The principal toxicities were transient grade 1-2 fevers and malaise in 5 pts and grade 1-2 hypotension in 2 pts. MDX-210 was biologically active at all doses. Substantial, peripheral blood monocytopenia occurred in all pts by 1 hr and resolved by 24 hr. Mild neutropenia occurred at 1 hr and resolved by 2 hr. There were no changes in platelets or erythrocytes. Plasma TNFalpha increased to as high as 500 pg/ml in 5 of the 6 pts tested within 1-8 hr and resolved by 24 hr. Dose-dependent in vivo binding of MDX-210 to CD64 was observed with up to 80% saturation at 1 hr and significant binding for greater than or equal to 24 hr. No changes in T cell, B cell or NK cell subsets were observed 4 or 24 hr after treatment. MDX-210 is immunologically active at non-toxic doses and is a candidate for immunotherapy of Her-2/neu expressing tumors.

L102 ANSWER 2 OF 22 CANCERLIT
AN 94602995 CANCERLIT
TI Phase I trial of a bispecific murine monoclonal antibody targeting c-erbB-2 and CD16 (Meeting abstract).
AU Weiner L M; Ring D I; Li W; Palazzo I E; Davey M; Rivera V; Alpaugh R K
CS Fox Chase Cancer Center, Philadelphia, PA 19111
SO Proc Annu Meet Am Assoc Cancer Res, (1994). Vol. 35, pp. A1309. ISSN: 0197-016X.
DT (MEETING ABSTRACT)
(CLINICAL TRIAL, PHASE I)
(CLINICAL TRIAL)
FS ICDB
LA English
EM 9411
AB Bispecific monoclonal antibodies (**BsMAb**) can direct tumor lysis by effector cells via defined cytotoxic trigger molecules. The **BsMAb** 2B1 promotes c-erbB-2 tumor cell lysis by human NK cells and macrophages expressing **CD16** (ie, **FcgammaRIII**), and is effective in murine xenograft models. Neutrophils (PMN) express an isoform of **CD16** that does not trigger lysis. In a dose-escalating Phase I clinical trial, nine patients with c-erbB-2(+) tumors have been treated iv with 1 hr **BsMAb** infusions on days 1, 4, 5, 6, 7 and 8, at 1 mg/m2 (n=3) or 2.5 mg/m2 (n=6) 2B1 per dose. The MTD has not been reached. Treatment causes fevers, rigors, reversible neutropenia and loss of circulating monocytes and NK cells. Treatment also alters the distribution of 111In-labeled autologous leukocytes, with tumor localization noted in at least one patient. Circulating 2B1 retains its dual binding characteristics. Peak levels of 240-2260 ng/ml murine IgG have been detected, with binding to circulating and peritoneal PMN, NK cells and mononuclear phagocytes. One clinical response has been observed in a patient with chest wall recurrence

of breast cancer. The binding of this **BsMAb** to **CD16**-expressing leukocytes has potent biological effects which may be exploited at higher, tumor-binding 2B1 doses.

L102 ANSWER 3 OF 22 CANCERLIT
AN 94600974 CANCERLIT
TI Phase I trial of 2B1, a bispecific murine monoclonal antibody targeting c-erbB-2 and CD16 (Meeting abstract).
AU Weiner L M; Ring D; Li W; Palazzo I E; Davey M; Rivera V; Alpaugh R K
CS Fox Chase Cancer Center, Philadelphia, PA 19111
SO Proc Annu Meet Am Soc Clin Oncol, (1994). Vol. 13, pp. A978. ISSN: 0736-7589.
DT (MEETING ABSTRACT)
(CLINICAL TRIAL, PHASE I)
(CLINICAL TRIAL)
FS ICDB
LA English
EM 9409
AB Bispecific monoclonal antibodies (**BsMAb**) can direct tumor lysis by effector cells via defined cytotoxic trigger molecules without competition by host IgG. The **BsMAb** 2B 1 promotes c-erbB-2 tumor cell lysis by human NK cells and macrophages expressing **CD16** (ie, **Fc** gamma RIII), and is effective in a murine xenograft model. Neutrophils (PMN) abundantly express an isoform of **CD16** that does not trigger tumor lysis. In a dose-escalating Phase I clinical trial, eleven patients with c-erbB-2(+) tumors and ECOG PS = 0 or 1 have been treated iv with 1 hr **BsMAb** infusions on days 1, 4, 5, 6, 7 and 8, at 1 mg/m2 (n=3), 2.5 mg/m2 (n=6) or 5.0 mg/m2 (n=2) 2B1 per dose. The MTD has not been reached. The severity of fevers, rigors, transient Grade 4 neutropenia and the loss of circulating monocytes and NK cells are not dose-dependent. Peak serum levels of TNF (2400 pg/ml), IL-6 (9000 pg/ml), IL-8 (11 pg/ml) and elastase (600 ng/ml) are found 1-4 hr following the start of **BsMAb** infusion. Tumor localization of 111In-labeled autologous leukocytes has been noted. Dose-related peak serum levels of 240-2260 ng/ml murine IgG have been detected, as has HAMA. Circulating 2B1 retains its dual binding characteristics and can be found on circulating and peritoneal PMN, NK cells and mononuclear phagocytes by flow cytometry. Treatment enhances peripheral blood NK activity and LAK precursors, resulting in augmented in vitro growth inhibition of c-erbB-2(+) SK-OV-3 cells. A clinical response was observed in a patient with an evaluable chest wall recurrence of breast cancer. This first clinical trial of a **BsMAb** targeting tumor and **CD16** has demonstrated potent biological effects which may be exploited at higher, tumor-binding 2B1 doses. The role of PMN in promoting these effects requires examination.

L102 ANSWER 4 OF 22 CANCERLIT
AN 94600969 CANCERLIT
TI Phase Ia/Ib trial of bispecific monoclonal antibody (BsAb) MDX-210 (anti-HER-2/neu x ANTI-CD64) for breast or ovarian cancers that over

express HER-2/neu (Meeting abstract).
AU Valone F H; Kaufman P A; Fanger M W; Guyre P M; Memoli V; Lewis L D
CS Dartmouth-Hitchcock Medical Center, Lebanon, NH 03756
SO Proc Annu Meet Am Soc Clin Oncol, (1994). Vol. 13, pp. A973.
ISSN: 0736-7589.
DT (MEETING ABSTRACT)
(CLINICAL TRIAL, PHASE I)
(CLINICAL TRIAL)
FS ICDB
LA English
EM 9409
AB MDX-210 is a Fab' x Fab' **BsAb** constructed by chemically cross-linking Fab' mAb 520C9 (anti-Her-2/neu) and Fab' mAb22 (anti-CD64). CD64 is a high affinity **Fc** receptor for IgG. Her-2/neu is a proto-oncogene that is over expressed in 20-30% of breast and ovarian cancers. MDX-210 effectively directs cytotoxicity and phagocytosis of tumor cells by human monocyte-derived macrophages and IFN gamma-treated neutrophils in vitro. MDX-210 triggers release of TNF alpha by macrophages in the presence, but not in the absence, of HER-2/neu expressing target cells. The trial's endpoints are evaluation of toxicity, determination of the MTD and pharmacokinetic profile, and biological efficacy. Patients with stage IV breast cancer or stage III or IV ovarian cancer that is resistant to standard therapy and expresses Her-2/neu are eligible for treatment. Three patients at each dose level of 0.35, 1.0, 3.5 and 7.0 mg/m2 (given iv once at 6 mg/hr) have been treated. Treatment was well tolerated with only grade 1-2 toxicities being observed. The principal toxicities were transient fevers and malaise in 9 pts and mild hypotension in 9 pts. MDX-210 was biologically active at all doses. Substantial, monocytopenia occurred within 1 hr and resolved by 24 hr. Mild neutropenia occurred at 1 hr and resolved by 2 hr. Platelets and erythrocytes did not change. Plasma TNF alpha increased to as high as 250 pg/ml within 1-8 hr and resolved by 24 hr after treatment. IL-1 alpha, IL-1 beta, and IL-6 did not increase. Dose-dependent in vivo binding of MDX-210 to CD64 on circulating monocytes was observed with up to 80% saturation of CD64 at 1 hr and significant binding for greater than or equal to 24 hr. No changes in T cell, B cell or NK cell subsets were observed. MDX-210 plasma concentrations were measured in 3 patients who received 3.5 mg/m2. Pharmacokinetic analysis revealed that the data best fitted a single open one compartment model. Cmax ranged from 0.88 to 1.02 ug/ml; t1/2 ranged from 11.4 to 12.5 hr; CL and Vd estimates ranged from 0.32 to 0.35 L/hr and 5.5 to 6.2 L, respectively. MDX-210 is a novel bispecific monoclonal antibody that is immunologically active at tolerable doses and is a candidate for immunotherapy of tumors that express Her-2/neu.

L102 ANSWER 5 OF 22 BIOSIS COPYRIGHT 1996 BIOSIS DUPLICATE 1
AN 94:259714 BIOSIS
DN 97272714
TI Potentiation of long-term-cultured lymphokine-activated killer cell cytotoxicity against small-cell lung carcinoma by anti-CD3 X anti-(tumor-associated antigen) bispecific antibody.

AU Azuma A; Yagita H; Okumura K; Kudoh S; Niitani H
CS 4th Dep. Internal Med., Nippon Med. Sch., 1-1-5 Sendagi, Bunkyo-ku, Tokyo 113, JAP
SO Cancer Immunology Immunotherapy 38 (5). 1994. 294-298. ISSN: 0340-7004
LA English
AB Lymphokine-activated killer (LAK) cells exhibit a potent cytotoxicity to malignant cells in vitro. However, a satisfactory effect has not been obtained in many clinical studies except for a few cases. One of the most important reasons why cytolytic activity could not be exhibited in vivo is that LAK cells do not accumulate in the tumor tissue because of a lack of specificity. In the present study, we show the effect of a bispecific antibody (**bsAb**) on the accumulation of LAK cells around the small-cell lung carcinoma (SCLC) cell and the subsequent enhancement of LAK cell cytotoxicity against SCLC. When short-term(4 days)-cultured LAK cells were used, OKT3 times LU246 **bsAb**, which direct CD3+ T-LAK cells to the target cell. induced a similar level of cytotoxicity to that induced by 3G8 times LU246 **bsAb**, which directs **CD 16+** LAK cells. Longterm(21 days)-cultured LAK cells exhibited a reduced spontaneous cytotoxicity but retained high cytotoxic activity, which could be directed by OKT3 times LU246 or 3G8 times LU246 **bsAb**. The inhibitory effect of LAK cells on tumor cell clonogenicity in soft agar was also enhanced by both **bsAb**. These results indicate that application of the immunotherapy.

L102 ANSWER 6 OF 22 EMBASE COPYRIGHT 1996 ELSEVIER SCI. B.V.
AN 94339998 EMBASE
TI Evaluation of Fc.gamma. receptor mediated T-cell activation by two purified CD3 x CD19 bispecific monoclonal antibodies with hybrid Fc domains.
AU Haagen I.-A.; Geerars A.J.G.; Bast E.J.E.G.; De Gast G.C.; Van De Winkel J.G.J.; De Lau W.B.M.
CS Department of Immunology, University Hospital, PO Box 85500, 3508 GA Utrecht, Netherlands
SO THER. IMMUNOL., (1994) 1/5 (279-287).
ISSN: 0967-0149 CODEN: THIMEY
CY United Kingdom
DT Journal
FS 016 Cancer
026 Immunology, Serology and Transplantation
037 Drug Literature Index
LA English
SL English
AB Two bispecific monoclonal antibodies (**BsAb**), differing in H chain isotype combination, were made for treatment of B-cell leukaemia/lymphoma; QAI-2, CD3-mouse IgG1 x CD19-mouse-IgG2a and QAI-3, CD3-mouse-IgG1 x CD19-mouse-IgG2b. Both purified **BsAb** proved equally effective for their ability to target pre-activated T cells towards CD19 positive tumour cells. In T-cell proliferation assays, the capacity of **Fc**.gamma.RIa (CD64), **Fc** .gamma.RIIa-R131 and **Fc**.gamma.RIIa-H131 (CD32) transfected

fibroblasts was tested to present the **BsAb**. The **BsAb** combining mouse (m) IgG1 and mIgG2a promoted T-cell activation in combination with the **Fc**.gamma.RIa transfectant; the mIgG1m-IgG2b **BsAb** was only marginally active. Both **BsAb** could not induce T-cell activation when presented by either of the **Fc**.gamma.RIIa transfectants. Similar results were obtained using PBMC cultures, containing **Fc**.gamma.RIa+/**Fc**.gamma.RIIa+ monocytes as accessory cells. The importance of **Fc**.gamma.R-dependent **BsAb**-mediated T-cell activation emerged from experiments with T cells and CD19 positive B-cell lines, showing that cross-linking via CD19+ target cells alone did not induce T-cell proliferation. Therefore, **BsAb** with functionally different **Fc** domains represent alternative strategies in **BsAb** therapy, the efficacy of which deserves to be compared in vivo.

L102 ANSWER 7 OF 22 BIOSIS COPYRIGHT 1996 BIOSIS DUPLICATE 2
AN 94:322419 BIOSIS
DN 97335419
TI Alterations of the blood-aqueous barrier in retinitis pigmentosa: First results.
AU Kuechle M; Nguyen N X; Schalnus R; Freissler K; Luechtenberg M; Mueller M
CS Augenklinik Poliklinik Univ. Erlangen-Nuernberg, Schwabachanlage 6, D-91054 Erlangen, GER
SO Klinische Monatsblaetter fuer Augenheilkunde 204 (4). 1994. 211-216. ISSN: 0023-2165
LA German
AB Background: Clinical findings in patients with retinitis pigmentosa (RP) frequently include cystoid macular edema (CME) and vitreous pigment dusting (VPD) indicating alterations of the blood-ocular barriers. It was the aim of this study to determine whether alterations of the blood-aqueous barrier (**BAB**) are present in RP. Patients and methods: Aqueous flare was quantitatively determined in 56 eyes of 29 patients (mean age 36.4 +- 11.2 years) with RP using the noninvasive laser flare-cell photometer (Kowa **FC**-1000, Tokyo, Japan). All RP patients had common forms of RP with markedly reduced or nondetectable ERG. The presence of CME and VPD was determined semiquantitatively by biomicroscopy. Fifty-eight eyes of 58 normal controls (mean age 38.2 +- 8.5 y.) were also examined. Results: Aqueous-flare values were significantly higher in RP eyes (mean 9.65 +- 3.24 photon counts/ms, range 4.0-18.1) than in the normal control eyes (mean 3.89 +- 1.05, range 1.9-6.0, p lt 0.0001, Mann-Whytney test). Forty-eight of the 56 RP eyes (86%) showed increased flare values (gt 6.0 photon counts/ms). RP eyes with moderate to marked CME (n=11) had significantly higher flare values (mean 14.66 +- 1.92) than RP eyes without or with only questionable CME (8.52 +- 2.18, p lt 0,0001), and RP eyes with moderate to marked VPD (n=17) had significantly higher (12.05 +- 2.84) flare values than RP eyes without or with only minimal VPD (8.74 +- 2.22, p lt 0.0005). Conclusions: Our findings show that the majority of patients with RP have alterations of the **BAB** with consecutively increased aqueous protein concentrations. The

impairment of the **BAB** appears to be associated with CME and VPD. Measurement of aqueous flare in RP allows quantification of the impairment of the **BAB** and may be helpful in the future in choosing and monitoring patients with RP and CMP for possible antiinflammatory or antiedematous therapeutic measures.

L102 ANSWER 8 OF 22 BIOSIS COPYRIGHT 1996 BIOSIS DUPLICATE 3
AN 93:523471 BIOSIS
DN BA96:136878
TI CLUSTERED CD3-TCR COMPLEXES DO NOT TRANSDUCE ACTIVATION SIGNALS AFTER BISPECIFIC MONOCLONAL ANTIBODY-TRIGGERED LYSIS BY CYTOTOXIC T LYMPHOCYTES VIA CD3.
AU BLANK-VOORTHUIS C J A; BRAAKMAN E; RONTELTAP C P M; TILLY B C; STURM E; WARNAAR S O; BOLHUIS R L H
CS DEP. CLINICAL TURMOR IMMUNOL., DANIEL DEN HOED CANCER CENTER, PO BOX 5201, 3008 AE ROTTERDAM, NETH.
SO J IMMUNOL 151 (6). 1993. 2904-2914. CODEN: JOIMA3 ISSN: 0022-1767
LA English
AB Bispecific mAb (**bsmAb**) directed against the CD3/**FCR** complex and a tumor-associated Ag (TAA) induces CTL-mediated lysis of TAA+ target cells. We have investigated whether **bsmAb** -pretargeted CTL can enter multiple lytic cycles. **BsmAb** -pretargeted CTL retained **bsmAb**-targeted lytic capacity for at least 24 h when exposed to medium without TAA+ target cells. Exposure of **bsmAb**-pretargeted CTL to TAA+ target cells resulted in a rapid loss of **bsmAb**-targeted cytotoxicity of TAA+ or **Fc**-yR+ target cells, although the CTL retained surface **bsmAb**. Moreover, addition of rabbit anti-mouse Ig to these CTL did not induce calcium mobilization. These CTL still showed Ag-specific cytotoxicity and cytolysis of anti-CD3 mAb-expressing hybridoma cells. Readdition of **bsmAb** to CTL that had lost **bsmAb**-targeted cytotoxicity instantly restored the **bsmAb**-targeted lytic activity of the CTL. Hence, as in Ag-specific cytolysis, **bsmAb**-pretargeted CTL can enter multiple **bsmAb**-targeted cytolytic cycles. Surprisingly, exposure of **bsmAb**-pretargeted CTL to **FcyR**+ cells did not result in loss of **bsmAb** -targeted cytolysis of TAA+ cells. Fluorescence microscopic analysis revealed that **bsmAb**-mediated interaction with TAA+ cells, but not with **Fc**.gamma.R+ cells, resulted in clustering of **bsmAb**-pretargeted CD3/TCR complexes on the CTL surface. On the basis of the observed correlation between clustered **bsmAb** -pretargeted CD3/TCR complexes and loss of **bsmAb**-targeted cytotoxicity, we hypothesize that clustered CD3/TCR complexes can no longer transduce signals.

L102 ANSWER 9 OF 22 BIOSIS COPYRIGHT 1996 BIOSIS DUPLICATE 4
AN 93:480726 BIOSIS
DN BA96:114326
TI BINDING AND CYTOTOXICITY CHARACTERISTICS OF THE BISPECIFIC MURINE MONOCLONAL ANTIBODY 2B1.
AU WEINER L M; HOLMES M; RICHESON A; GODWIN A; ADAMS G P; HSIEH-MA S T; RING D B; ALPAUGH R K

CS FOX CHASE CANCER CENTER, 7701 BURHOLME AVE., PHILADELPHIA, PA 19111, USA.
SO J IMMUNOL 151 (5). 1993. 2877-2886. CODEN: JOIMA3 ISSN: 0022-1767
LA English
AB Bispecific monoclonal antibodies (**BsmAb**) with specificity for tumor Ag and effector cell trigger molecules have been shown to redirect the cytotoxicity of several peripheral blood mononuclear cell populations against relevant tumor. The **BsmAb**, 2B1, binds to the extracellular domain of the c-erbB-2 gene product of the HER2/neu protooncogene and to **CD16**. In this report, the binding and cytotoxic characteristics of 2B1 are presented. Maximal saturation binding of 2B1 to PBL and c-erbB-2 expressing SK-OV-3 cells occurred in the 1 .mu.g/ml concentration range. However, substantial lysis potentiation was observed at 1000-fold lower **BsmAb** concentrations. Optimal tumor lysis was obtained when the **BsmAb**, PBL, and target cells were continuously coincubated. When PBL were franked with 2B1, washed, and added to labeled targets, substantially less lysis was observed. These results suggest that the best way to therapeutically exploit the cytotoxic attributes of 2B1 may be to obtain continuous **BsmAb** exposure to tumor. Approaches based on franking of this **BsmAb** to PBL may not be warranted.

L102 ANSWER 10 OF 22 CANCERLIT
AN 93693790 CANCERLIT
TI Binding characteristics of the bispecific monoclonal antibody 2B1 (Meeting abstract).
AU Garcia de Palazzo I E; Richeson A; Weiner L M
CS Fox Chase Cancer Center, Philadelphia, PA 19111
SO Proc Annu Meet Am Assoc Cancer Res, (1993). Vol. 34, pp. A2847. ISSN: 0197-016X.
DT (MEETING ABSTRACT)
FS ICDB
LA English
EM 9308
AB Bispecific monoclonal antibodies (**BsMAb**) with specificity for selected tumor antigens and **CD16** (**Fc** gamma RIII) redirect the cytotoxicity of several peripheral blood mononuclear cell populations against relevant tumor. **BsMAb** 2B1 binds to the extracellular domains of c-erbB-2 and **CD16** . Peak binding of 2B1 to peripheral blood lymphocytes (PBL) expressing **Fc** gamma RIII and c-erbB-2(+) tumor cells occurs in the 1 ug/ml concentration range, even though peak lysis is seen at 10 ng/ml. 2B1 is not well-retained by human PBL at 37 C, and PBL franked with 2B1 do not promote tumor lysis as efficiently as when PBL and tumor are coincubated in the presence of ng/ml 2B1 concentrations. The expression of c-erbB-2 in 126 malignant tumors was investigated by immunohistology utilizing 2B1 and parent antibody 520C9. 49% of primary breast, lung, colorectal and ovarian carcinomas stained with 520C9 and 39% with 2B1, and metastases stained at least as well. 2B1 detects a high percentage of tumors overexpressing c-erbB-2, but 2B1 therapy strategies should focus on the delivery of **BsMAb** to tumor sites and not circulating

effectors.

L102 ANSWER 11 OF 22 BIOSIS COPYRIGHT 1996 BIOSIS DUPLICATE 5
AN 92:306388 BIOSIS
DN BA94:19538
TI COMPARATIVE EFFICIENCIES OF BISPECIFIC FAB'-GAMMA-2 AND CHIMERIC MOUSE-HUMAN IGG ANTIBODIES IN RECRUITING CELLULAR EFFECTORS FOR CYTOTOXICITY VIA FC-GAMMA RECEPTORS.
AU GREENMAN J; HOGG N; NIKOLETTI S; SLADE C; STEVENSON G; GLENNIE M
CS TENOVUS RES. LAB., GENERAL HOSP., TREMONA RD., SOUTHAMPTON, UK.
SO CANCER IMMUNOL IMMUNOTHER 34 (6). 1992. 361-369. CODEN: CIIMDN ISSN: 0340-7004
LA English
AB The three forms of **Fc**.gamma. receptor carried by monocytes (**Fc**.gamma.RI, II) and natural killer (NK) cells (**Fc** .gamma.RIII) are all capable of mediating cell lysis. Here we compare the use of F(ab'.gamma.)2 bispecific antibodies, specifically targetting individual **Fc**.gamma.R, and chimeric IgG mouse/human antibodies which are capable of targetting all **Fc** .gamma.R, for their ability to mediate target cell destruction. The derivatives are prepared by linking hinge sulphydryl residues via tandem thioether bonds, using a bismaleimide crosslinker: Fab' from an anti-**Fc**.gamma.R mAb linked to Fab' from a common anti-target mAb (**BsAb**), or Fab' from the common anti-target mouse antibody linked to human **Fc**.gamma. (FabFc or bisFabFc). All the derivatives targetting chick red blood cells gave efficient lysis, although different effector cell donors yielded differences in both the lytic levels achieved and the comparative efficiencies of derivatives. In contrast, significant lysis of the guinea pig lymphoblastic leukaemia, L2C, regularly resulted only via the anti-**Fc**.gamma.RIII **BsAb** and the chimeric derivatives. These results suggest that the chimeric, **Fc** -containing derivatives mediate tumour cell lysis principally through **Fc**.gamma.RIII on NK cells. This is in contrast to the situation which the chick red blood cells where the chimeric derivatives appear capable of lysing erythrocytes by utilizing either monocytes or NK cells, because significant (.apprxeq. 50%) lysis occurred with effector cell populations magnetically depleted through either **FcYrRIII** or **Fc**.gamma.RIII. A major difference between these two types of antibody derivative was their ability to function in the presence of high concentrations of normal human **Fc**.gamma.. The lysis mediated by **BsAb** reactive with **Fc**.gamma.RI or II was unaffected by the presence of human **Fc**.gamma. at 2.5 mg/ml (a concentration comparable with that yielded by IgG in plasma) whereas the **BsAb** recognizing **Fc**.gamma.RIII and all the **Fc**-containing derivatives were completely inhibited.

L102 ANSWER 12 OF 22 BIOSIS COPYRIGHT 1996 BIOSIS
AN 92:385344 BIOSIS
DN BR43:52294
TI EFFECTIVE TARGETING OF HUMAN LYMPHOCYTES AND MACROPHAGES TO TUMOR BY A BISPECIFIC MONOCLONAL ANTIBODY **BSMAB** BINDING HUMAN

C-ERBB-2 AND FC-GAMMA-RIII.
AU LEE G; WEINER L; RING D; HOLMES M; ALPAUGH K; GARCIA DE PALAZZO I
CS FOX CHASE CANCER CENT., PHILADELPHIA, PA. 19111.
SO 83RD ANNUAL MEETING OF THE AMERICAN ASSOCIATION FOR CANCER RESEARCH, SAN DIEGO, CALIFORNIA, USA, MAY 20-23, 1992. PROC AM ASSOC CANCER RES ANNU MEET 33 (0). 1992. 344. CODEN: PAMREA
DT Conference
LA English

L102 ANSWER 13 OF 22 CANCERLIT
AN 91675307 CANCERLIT
TI ACTIVATION AND EXPANSION OF T LYMPHOCYTES IN HOLLOW FIBER BIOREACTORS (MEETING ABSTRACT).
AU Bolhuis R L; Lamers C J; van de Griend R J; Ronteltap C P; Braakman E
CS Dept. of Immunology, Daniel den Hoed Cancer Center, PO Box 5201, 3008 AE Rotterdam, The Netherlands
SO Proc Annu Meet Am Assoc Cancer Res, (1991). Vol. 32, pp. A1640. ISSN: 0197-016X.
DT (MEETING ABSTRACT)
FS ICDB
LA English
EM 9110
AB Human peripheral blood lymphocytes were activated and expanded in parallel with PHA plus IL-2, using Acusyst hollow fiber technology and culture bags. The following parameters were analyzed: cell growth, growth kinetics, phenotype, cytolytic activity and cytolytic response capacity to bispecific monoclonal antibodies. PBL, separated on Ficoll Isopaque, were inoculated in hollow fiber cartridges activated with PHA (1 ug/ml) and expanded with IL-2 (1000 U/L). At day 14, lymphocytes were harvested and analyzed by FACSCAN and double staining. In experiment 1, lymphocytes expanded greater than 50-fold from 1 x 10(9) till 54 x 10(9), and 98% were CD3+, 53% were CD8+ and 44% were CD4+. Only 4% of the lymphocytes were CD 56+ and about 1% were CD3- CD16+ (NK). The cytolytic response capacity of anti-CD3 x antiovarian Ca bsab targeted lymphocyte population ranged in between 14-38% tumor cell lysis. Cellular cytolysis of K562 and Daudi ranged in between 8-23% and 18-70%, respectively. Both lytic activities were substantially increased following 4 hr incubation of the lymphocytes in medium supplemented with serum.

L102 ANSWER 14 OF 22 CANCERLIT
AN 91674366 CANCERLIT
TI BISPECIFIC ANTIBODY PROMOTION OF TUMOR LYSIS IS MEDIATED IN WHOLE BLOOD (MEETING ABSTRACT).
AU Weiner L M; Holmes M; Garcia dePalazzo I E
CS Fox Chase Cancer Center, Philadelphia, PA 19111
SO Proc Annu Meet Am Assoc Cancer Res, (1991). Vol. 32, pp. A1557. ISSN: 0197-016X.
DT (MEETING ABSTRACT)
FS ICDB
LA English

EM 9107
AB Bispecific murine monoclonal antibodies (**BsMAb**) targeting tumor and selected epitopes of **Fc** gamma receptors (**Fc** gamma R) are superior to conventional antitumor antibodies because very low **BsMAb** concentrations promote tumor lysis by **Fc** gamma RIII expressing effector cells in the presence of human serum containing **Fc** gamma R-binding immunoglobulins. If **BsMAb** are to be therapeutically useful, they must be able to exert their effects in whole blood, overcoming competition by human serum, circulating **Fc** gamma RIII, and polymorphonuclear leukocytes (PMNs), which express a nonmembrane-anchored isoform of **Fc** gamma RIII. **BsMAb** CL 158 (alpha CA 19-9 x alpha **Fc** gamma RIII) binds to the CA 19-9 expressing adenocarcinoma cell line SW948; the ability of this antibody to induce lysis of 51Cr-SW948 cells was tested in heparinized whole blood activated for 36-72 hr in 1000 U/ml IL-2. Lysis rose from 33.0 +/- 6.5% in the absence of antibody to 85.0 +/- 5.2% when 10 ng/ml CL 158 were added. When the IgG2a variant of CA 19-9 antibody was used, lysis averaged only 37%. In whole blood, multicellular human tumor spheroids composed of SW948 cells were infiltrated and completely disrupted by large granular lymphocytes (LGLs) only when the relevant **BsMAb** was added. Thus, CL 158 **BsMAb** uniquely redirects LGL cytotoxicity in whole blood to destroy organized tumor by circumventing competition from human immunoglobulin without interference from PMNs or circulating **Fc** gamma RIII. These properties support the rationale for **BsMAb** therapy.

L102 ANSWER 15 OF 22 CANCERLIT
AN 91674347 CANCERLIT
TI NEURAL CELL ADHESION MOLECULE (N-CAM) INTERACTIONS PROMOTE LYSIS OF TUMOR BY BISPECIFIC ANTIBODY REDIRECTED LYMPHOCYTES (MEETING ABSTRACT).
AU Garcia-dePalazzo I; Gercel-Taylor C; Kitson J; Adams S; Weiner L M
CS Fox Chase Cancer Center, Philadelphia, PA 19111
SO Proc Annu Meet Am Assoc Cancer Res, (1991). Vol. 32, pp. A1538. ISSN: 0197-016X.
DT (MEETING ABSTRACT)
FS ICDB
LA English
EM 9110
AB Bispecific monoclonal antibodies (**BsMAb**) prepared by somatic cell fusion bind monovalently to their targets, yet are extremely potent enhancers of target cell lysis by relevant effector cells at low antibody concentrations. To investigate the mechanisms underlying this efficiency, we studied the ability of selected antibodies to modulate potentiation of tumor lysis by a **BsMAb** (CL158) that targets **Fc**-gamma RIII-expressing cells to malignant cells expressing CA19-9 antigen. Leu 19 antibody reversed antibody-dependent, but not antibody-independent lysis of 51Cr-labeled SW948 cells by IL-2 activated PBLs in concentration-dependent fashion. Leu 19 binds to CD56, a neural cell adhesion molecule (N-CAM) isoform expressed by

large granular lymphocytes (LGLs). The effects of Leu 19 on **BsMAb** promotion of lysis were not due to competition for binding to **FC**-gamma RIII, and were clearly due to inhibition of binding between LGLs and SW948 cells. Leu 19 did not inhibit antibody-dependent lysis by the monospecific, bivalent IgG2a variant of CA19-9 antibody, and had no effects on **BsMAb** -mediated lysis of N-CAM negative tumor cell lines. The efficient lysis promoted by **BsMAb** may be due to the ability of these proteins to promote closer apposition of effectors and targets, permitting engagement of diverse CAM that are not typically involved in antibody-dependent lysis.

L102 ANSWER 16 OF 22 MEDLINE DUPLICATE 6
AN 91020376 MEDLINE
TI The mechanism of anti-CD3 monoclonal antibodies. Mediation of cytolysis by inter-T cell bridging.
AU Wong J T; Eylath A A; Ghobrial I; Colvin R B
CS Department of Pathology, Massachusetts General Hospital, Boston..
NC R29-AI27050 (NIAID)
PO1-HL18646 (NHLBI)
T32-CA09216 (NCI)
SO TRANSPLANTATION, (1990 Oct) 50 (4) 683-9.
Journal code: WEJ. ISSN: 0041-1337.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals; Cancer Journals
EM 9101
AB OKT3, an anti-CD3 MAB, depletes T cells in vivo and is among the most potent inhibitors of acute allograft rejection. The mechanism of this inhibition is unknown. The present studies investigate whether anti-CD3 antibodies have the ability to crosslink CD3 on two different cells and induce TCR-dependent antibody-bridged cell-mediated cytolysis (TCR-ABCMC) between T cells. Two different anti-CD3 antibodies (OKT3 and CD3,3) and OKT3 F(ab')2 were all highly effective in inducing cytolysis of CD8+ and CD4+ T cells by CD8+ T cells, and CD8+ T cells by CD4+ T cells. Monovalent OKT3 Fab was 25-125-fold less potent than OKT3 F(ab')2. Monovalent CD3,X was totally ineffective. The necessity for intercellular bridging was evidenced by the observation that an anti-CD3:anti-CD4 (CD3,4) bispecific MAB (**BSMAB**) was effective in mediating lysis of CD4+ but not CD8+ T cells by CD8+ T cells. These studies indicate that neither **FcR**-mediated ADCC nor complement fixation is necessary for bivalent anti-CD3 MAB to lyse T cells. Inter-T cell TCR-ABCMC may be particularly effective in inflammatory tissues, such as rejecting allografts and autoimmune diseases, in which numerous cytolytic T lymphocytes are present in close association with other T cells.

L102 ANSWER 17 OF 22 BIOSIS COPYRIGHT 1996 BIOSIS DUPLICATE 7
AN 87:463087 BIOSIS
DN BA84:108527
TI BI-SPECIFIC MONOCLONAL ANTIBODIES SELECTIVE BINDING AND COMPLEMENT

FIXATION TO CELLS THAT EXPRESS TWO DIFFERENT SURFACE ANTIGENS.
AU WONG J T; COLVIN R B
CS IMMUNOPATHOL. UNIT, COX 5, MASS. GEN. HOSP., BOSTON, MASS. 02114, USA.
SO J IMMUNOL 139 (4). 1987. 1369-1374. CODEN: JOIMA3 ISSN: 0022-1767
LA English
AB A new dimension of immunotherapeutic selectivity might be achieved if antibodies could distinguish cells that co-express two different surface antigens. Bi-specific monoclonal antibodies (**BSMAB**) with two different antigen combining sites that share a common **Fc** region theoretically might have such a potential. Two such **BSMAB** were produced by hybrid-hybridoma clones prepared by fusion of pre-existing hybridomas and were purified by isoelectric focusing. CD3,4, (IgG2a, IgG2b) recognizes the T cell surface antigens CD3 and CD4, and CD3,8 (IgG2a, IgG2a) recognizes CD3 and CD8. These **BSMAB** promote complement-mediated lysis of target cells that bear both surface antigens 25 to 3125 times more efficiently than those that express only one of the antigens. This selectivity results from the increased avidity of these antibodies for cells with both antigens, as reflected by the increased surface immunoglobulin concentration detected by flow cytometry. It was also demonstrated that there exists a threshold surface immunoglobulin density necessary for antibody-dependent complement-mediated cytotoxicity microtiter assays for the various IgG antibodies tested in both bivalent and monovalent binding. These results support the associative model of IgG-mediated complement fixation.

L102 ANSWER 18 OF 22 BIOSIS COPYRIGHT 1996 BIOSIS DUPLICATE 8
AN 85:251436 BIOSIS
DN BA79:31432
TI ANALYSIS OF THE REACTIVITY OF 4 ANTI-MOUSE IMMUNOGLOBULIN M ALLOTYPE ANTIBODIES WITH MU-PLUS B LINEAGE CELLS AT VARIOUS STAGES OF DIFFERENTIATION.
AU VELARDI A; KUBAGAWA H; KEARNEY J F
CS 224 TUMOR INSTITUTE, UNIVERSITY OF ALABAMA IN BIRMINGHAM, UNIVERSITY STATION, BIRMINGHAM, ALA. 35294.
SO J IMMUNOL 133 (4). 1984. 2098-2103. CODEN: JOIMA3 ISSN: 0022-1767
LA English
AB Two rat monoclonal antibodies (Mab) (Bet-1 and 331.12), a mouse Mab (AF6-78.25) and an alloantiserum (SJA anti-**BAB**/14) were used in 2-color immunofluorescence analysis and enzyme-linked immunosorbent assays (ELISA) to examine the expression of IgM allotypes at various stages of mouse B cell development, especially at the pre-B cell stage. In agreement with previous findings, these antibodies displayed the patterns of allotypic reactivity with IgM+ B cells and plasma cells from the appropriate strains of mice: Bet-1 antibody is specific for a allotype, AF6-78.25 and SJA anti-**BAB**/14 for b allotype, and 331.12 for both a and b allotypes but not e allotype. These antibodies did not react with isolated .mu.-H chains prepared from secreted molecules or synthesized in situ by normal pre-B cells or pre-B cell-derived hybridomas. The requirement of L chain participation in the expression of IgM allotypic determinant(s) was additionally suggested by analysis of a

fetal liver-derived hybridoma (F1-26-11) that secretes IgM heteromolecules composed of C57BL/6-derived .mu.-chain and BALB/c-dervied .kappa.-chains. In contrast to the nonreactivity with C57BL/6 .mu.-only pre-B cell-derived hybridomas, all 3 antibodies with specificity for the b allotype (AF6-78.25, SJA anti-**BAB** /14 and 331.12) reacted with this particular hybridoma. After treating surface IgM molecules on B lymphocytes with papain to cleave Fab fragments, the b allotype reactivity of AF6-78.25 mouse Mab, but not 331.12 rat Mab, disappeared, whereas the a allotype reactivities of 2 rat Mab (Bet-1, 331.12) were not altered. Thus, the IgM allotypic epitopes defined by these antibodies appear to be sterically dependent on the assembly of the whole IgM molecules, and rat Mab 331.12 and mouse Mab AF6-78.25 define 2 separate b allotype specificities, one on the **Fc** portion and the other on the Fab portion of the IgM molecules, respectively.

L102 ANSWER 19 OF 22 LIFESCI COPYRIGHT 1996 CSA
AN 84:75876 LIFESCI
TI Regulation of antibody release by naturally occurring anti-immunoglobulins in cultures of pokeweed mitogen-stimulated human peripheral blood lymphocytes.
AU Birdsall, H.H.; Rossen, R.D.
CS Immunol. Res. Lab., Veterans Adm. Hosp., 2002 Holcombe Blvd., Houston, TX 77211, USA
SO J. IMMUNOL., (1984) vol. 133, no. 3, pp. 1257-1264.
DT Journal
FS F
LA English
SL English
AB Immunoregulatory influences of human anti-immunoglobulins (anti-Ig) were studied in cultures of peripheral blood lymphocytes (PBL) from 11 normal donors. Pokeweed mitogen (PWM)-stimulated PBL released anti-Ig specific for Fab' or **Fc** fragments of IgG, often within the first 24 to 72 hr in vitro. PBL that released more than 1 ng/ml IgM anti-Fab' during the first 72 hr in vitro ultimately produced significantly less antibody (AB) by the 12th day than PBL that released no detectable IgM anti-**Bab'** during the first 3 days in culture. Adding affinity-purified human anti-Fab' to PWM-stimulated PBL also suppressed the later Ab release by these cells. Suppression was polyclonal, affecting IgM anti-**Fc**, IgM anti-Fab', and IgM anti-tetanus toxoid Ab, and was directly dependent on the quantity of anti-Fab' added. These observations suggest that anti-Ig may exert a significant immunoregulatory role in man that can override to some extent the T cell-dependent stimulus for polyclonal B cell activation provided by PWM.

L102 ANSWER 20 OF 22 BIOSIS COPYRIGHT 1996 BIOSIS
AN 82:198533 BIOSIS
DN BA73:58517
TI FEED CONSUMPTION AND PERFORMANCE OF DAIRY COWS WITH ALTERNATE FEEDINGS OF GRASS SILAGE AND MAIZE SILAGE.
AU KIRCHGESSNER M; SCHWARZ F J; LINDNER H P
CS INST. ERNAEHRUNGSPHYSIOL., TECHNISCHE UNIV. MUENCHEN, D-8050

FREISING-WEIHENSTEPHAN, FRG.
SO ANIM FEED SCI TECHNOL 6 (4). 1981. 337-346. CODEN: AFSTDH ISSN: 0377-8401
LA English
AB A feeding experiment was conducted with 10 dairy cows of the Fleckvieh breed and the cross Red Holstein Friesian .times. Fleckvieh, to study whether feeding with grass silage at the morning meal and maize silage at the evening meal (treatment B: alternating forage allocation) affects forage intake and milk production, in comparison with combined feeding with these 2 silages at each meal (treatment A). To prevent a selective forage consumption in treatment A, the 2 silages were given as a homogeneous mixture of nearly equal portions (51.6% maize silage, 48.4% grass silage) of dry matter (DM). The experiment was of switch-back design, with the treatment sequences ABA and **BAB**, and 3 experimental periods of 6 wk. The daily forage consumption averaged 12.3 kg DM when the silages were given as a mixture was significantly higher than the total forage consumption of 11.8 kg DM ($P < 0.05$) during the alternating allocation of the silages. In treatment B, daily uptake of maize silage (7.10 kg DM) was greater than that of grass silage (4.70 kg DM/day). Variation between cows in forage intake was significantly higher in this treatment than in treatment A. Average daily milk yield for treatment A was 18.75 kg with 3.84% fat and 3.70% protein, and 18.10 kg with 3.76% fat and 3.68% protein for treatment B. Production was significantly higher ($P < 0.05$), by 0.65 kg milk or 0.90 kg **FCM**, for treatment A.

L102 ANSWER 21 OF 22 CANCERLIT
AN 79706535 CANCERLIT
TI CHARACTERIZATION OF A SPONTANEOUS MURINE B CELL LEUKEMIA (BCL(1)). I. CELL SURFACE EXPRESSION OF IGM, IGD, IA, AND FCR.
AU Knapp M R; Jones P P; Black S J; Vitetta E S; Slavin S; Strober S
CS Howard Hughes Medical Inst. Lab., Stanford Univ. Medical Center, Stanford, CA, 94305
SO J Immunol, (1979). Vol. 123, No. 3, pp. 992-999. ISSN: 0022-1767.
DT Journal; Article; (JOURNAL ARTICLE)
FS CARC
LA English
EM 7911
AB The surface marker expression of a spontaneous BALB/c B lymphocyte leukemia (BCL(1)) was examined. The tumor cells bore surface immunoglobulins that included both mu- and delta-chains associated with the gamma light chain. The presence of IgD with BALB/c (Iga) allotypic determinants was demonstrated on the surface of tumor cells growing in **BAB**/14 (Igb) animals, demonstrating the endogenous production of IgD by BCL1 cells. Alloantigens coded for within the murine H-2 complex, including H-2D, H-2K, and I-region products, were identified on the tumor cells. Although normal B lymphocytes are thought to express products coded for within both I-A and I-E subregions, the BCL1 expressed only normal amounts of I-E subregion products. In addition, H-2 and Ia antigens revealed by 2-dimensional gel electrophoresis exhibited an abnormal pattern of

post-translational modifications. The **Fc**, but not the complement receptor (CR), was present on the surface of tumor cells. The presence of several B cell surface markers suggests that BCL1 is derived from the B lymphocyte lineage. Since surface IgD and Ia antigens are first seen on mouse cells around the time of birth, after which they are found on most B lymphocytes, the present findings suggest that the BCL1 tumor represents a later differentiative stage than murine B lymphocyte tumors previously described. The absence of CR may be explained by the immaturity of the BCL1 cells, the restriction of cells to a CR-negative subpopulation, or to loss of a differentiated function by this cell line. (41 Refs)

L102 ANSWER 22 OF 22 MEDLINE
AN 76061535 MEDLINE
TI The binding of lanthanides to non-immune rabbit immunoglobulin G and its fragments.
AU Dower S K; Dwek R A; McLaughlin A C; Mole L E; Press E M; Sunderland C A
SO BIOCHEMICAL JOURNAL, (1975 Jul) 149 (1) 73-82.
Journal code: 9YO. ISSN: 0006-2936.
CY ENGLAND: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 7603
AB The binding of Gd(III) to rabbit IgG (immunoglobulin G) and the Fab (N-terminal half of heavy and light chain), (**Bab'**)2 (N-terminal half of heavy and light chains joined by inter-chain disulphide bond), **Fc** (C-terminal half of heavy-chain dimer)and pFc' (C-terminal quarter of heavy-chain dimer) fragments was demonstrated by measurements of the enhancement of the solvent-water proton relaxation rates in the appropriate Gd(III) solutions. At pH 5.5 there are six specific Gd(III)-binding sites on the IgG. These six sites can be divided into two classes; two very 'tight' sites on the **Fc** fragment (Kd approx. 5 muM) and two weaker sites on each Fab region (Kd approx. 140 muM). Ca(II) does not apparently compete for these metal-binding sites. The metal-binding parameters for IgG can be explained as the sum of the metal binding to the isolated Fab and **Fc** fragments, suggesting that there is no apparent interaction between the Fab and **Fc** regions in the IgG molecule. The binding of Gd(III) to Fab and **Fc** fragments was also monitored by measuring changes in the electron-spin-resonance spectrum of Gd(III) in the presence of each fragment and also by monitoring the effects of Gd(III) on the protein fluorescence at 340 nm (excitation 295 nm). The fluorescence of Tb(III) solutions of 545 nm (excitation 295 nm) is enhanced slightly on addition of Fab or **Fc**.

FILE 'CA' ENTERED AT 15:55:23 ON 02 APR 96

```
FILE 'CAPLUS' ENTERED AT 15:55:23 ON 02 APR 96
USE IS SUBJECT TO THE TERMS OF YOUR CUSTOMER AGREEMENT
COPYRIGHT (C) 1996 AMERICAN CHEMICAL SOCIETY (ACS)


L57            10 SEA FILE=CA "RING D"/AU OR "RING D F"/AU OR "RING DAVID F
                  "/AU OR "RING DAVID FRANCIS"/AU

=> s (divalen? or di valen? or bivalen? or bi valen?)(l)(fc? or cd16 or cd
16)
L103          254 FILE CA
L104          258 FILE CAPLUS

TOTAL FOR ALL FILES
L105          512 (DIVALEN? OR DI VALEN? OR BIVALEN? OR BI VALEN?)(L)(FC? OR
                  CD16 OR CD 16)

=> s l105 not (l21 or l67)
L106          252 FILE CA
L107          256 FILE CAPLUS

TOTAL FOR ALL FILES
L108          508 L105 NOT (L21 OR L67)

=> s l105(l)antigen?
L109           46 FILE CA
L110           47 FILE CAPLUS

TOTAL FOR ALL FILES
L111           93 L105(L) ANTIGEN?

=> s l111 not (l21 or l67)
L112           44 FILE CA
L113           45 FILE CAPLUS

TOTAL FOR ALL FILES
L114           89 L111 NOT (L21 OR L67)

=> s ran=,1994
ENTER LOGIC EXPRESSION, QUERY NAME, OR (END):l114
L115           42 FILE CA
L116           42 FILE CAPLUS

TOTAL FOR ALL FILES
L117           84 L114

=> s l117 and (bind? or bound)
L118           28 FILE CA
L119           28 FILE CAPLUS

TOTAL FOR ALL FILES
L120           56 L117 AND (BIND? OR BOUND)
```

signal transduction by the DNP Ag is halted. These results suggest a form of cellular memory for the desensitized state.

L122 ANSWER 3 OF 28 CA COPYRIGHT 1996 ACS DUPLICATE 3
AN 119:268617 CA
TI Inhibition of interferon-.gamma. by an interferon-.gamma. receptor immunoadhesin
SO Immunology (1993), 79(4), 594-9
CODEN: IMMUAM; ISSN: 0019-2805
AU Haak-Frendscho, M.; Marsters, S. A.; Chamow, S. M.; Peers, D. H.; Simpson, N. J.; Ashkenazi, A.
PY 1993
AB Interferon-.gamma. (IFN-.gamma.) is an important cytokine which regulates inflammatory and immune response mechanisms. IFN-.gamma. enhances the presentation and recognition of **antigens** by inducing the expression of major histocompatibility complex (MHC) proteins, by activating effector T cells and mononuclear phagocytes, and by modulating Ig prodn. and class selection in B cells. Inappropriate prodn. of IFN-.gamma. has been implicated in the pathogenesis of several autoimmune and inflammatory diseases and in graft rejection. Here, the authors describe a recombinant inhibitor of IFN-.gamma., termed murine IFN-.gamma. receptor immunoadhesin (mIFN-.gamma.R-IgG). The authors constructed this immunoadhesin by linking the extracellular portion of the mouse IFN-.gamma.R to the hinge and **Fc** region of an IgG1 heavy chain. Murine IFN-.gamma.R-IgG is secreted by transfected cells as a disulfide-bonded homodimer which **binds** IFN-.gamma. **bivalently**, with high affinity, and in a species-specific manner. In vitro, mIFN-.gamma.R-IgG can block mIFN-.gamma.-induced antiviral activity and expression of the class I MHC **antigen** H-2Kk in cultured cells. In vivo, mIFN-.gamma.R-IgG can block the function of the endogenous mIFN-.gamma. in mouse models of infection with Listeria monocytogenes and of contact sensitivity. Thus, mIFN-.gamma.R-IgG is an effective and specific inhibitor of mIFN-.gamma. both in vitro and in vivo. In general, IFN-.gamma. receptor immunoadhesins may be useful for investigating the biol. functions of IFN-.gamma. as well as for preventing deleterious effects of IFN-.gamma. in human disease.

L122 ANSWER 4 OF 28 CA COPYRIGHT 1996 ACS DUPLICATE 4
AN 120:161368 CA
TI Modulation of cell surface antigens and regulation of phagocytic activity mediated by CD11b in the monocyte-like cell line U937 in response to lipopolysaccharide
SO Tissue Antigens (1993), 42(3), 125-32
CODEN: TSANA2; ISSN: 0001-2815
AU Ikewaki, Nobunao; Tamauchi, Hidekazu; Inoko, Hidetoshi
PY 1993
AB Modulation of the cellular **antigens** and regulation of the phagocytic activity of the monocyte-like cell line U937 after culture with lipopolysaccharide (LPS) were investigated. CD14 expression was induced on the surface of the U937 cells after 48 h of culture with LPS and then they became adhesive with numerous

filamentous filopodia extruded on the cell surface, exhibiting enhanced expression of differentiation markers **CD16** and CD23. However, no induction or enhancement of the cell surface expression was obsd. with respect to CD11b, CD18, HLA-A, B, C, HLA-DR, DQ, DP or CD57. These U937 cells also acquired the ability to produce superoxide anions and to phagocytose the Salmonella enteritidis strain, 116-54. This phagocytosis was inhibited by the anti-CD11b monoclonal antibodies, but not by antibodies to CD14, **CD16**, CD18, CD23, HLA-A, B, C or HLA-DR. Thus, the phagocytic activity against Salmonella enteritidis 116-54 induced by LPS is mediated mainly via the CD11b mol., but is not assocd. with the increased expression of CD11b. Puromycin and cycloheximide, inhibitors of protein synthesis, or a **divalent** cation-chelating agent, EDTA, completely inhibited this phagocytic activity. Interestingly, EDTA suppressed specifically the CD11b expression on the U937 cells cultured with LPS. No phagocytic activity was induced when the U937 cells cultured with LPS were incubated at 4.degree., but restored to the control level when shifted up to 37.degree..

L122 ANSWER 5 OF 28 CA COPYRIGHT 1996 ACS DUPLICATE 5

AN 117:5731 CA

TI Aggregation of IgE-receptor complexes on rat basophilic leukemia cells does not change the intrinsic affinity but can alter the kinetics of the ligand-IgE interaction

SO Biochemistry (1992), 31(23), 5350-6
CODEN: BICHAW; ISSN: 0006-2960

AU Posner, Richard G.; Lee, Benjamin; Conrad, Daniel H.; Holowka, David; Baird, Barbara; Goldstein, Byron

PY 1992

AB The aggregation of IgE anchored to high-affinity **Fc** .epsilon. receptors on rat basophilic leukemia (RBL) cells by multivalent **antigens** initiates transmembrane signaling and ultimately cellular degranulation. Previous studies have shown that the rate of dissocn. of **bivalent** and multivalent DNP ligands from RBL cells sensitized with anti-DNP IgE decreases with increasing ligand incubation times. One mechanism proposed for this effect is that when IgE mols. are aggregated, a conformational change occurs that results in an increase in the intrinsic affinity of IgE for **antigen**. This possibility was tested by measuring the equil. const. for the **binding** of monovalent DNP-lysine to anti-DNP IgE under two conditions, where the cell-**bound** IgE is dispersed and where it has been aggregated into visible patches on the cell surface using anti-IgE and a secondary antibody. No difference in the equil. const. in these two cases was obsd. The rate was also measured of dissocn. of a monovalent ligand from cell surface IgE under these two conditions. Whereas the affinity for monovalent ligand is not altered by IgE aggregation, evidently the rate of ligand dissocn. from IgE in clusters is slower than the rate of ligand dissocn. from unaggregated IgE. These results are discussed in terms of recent theor. developments concerning effects of receptor d. on ligand **binding** to cell surfaces.

acetone fixation but no expression was seen after paraformaldehyde treatment, whereas in nasal polyps, similar expression was obsd. with both fixatives. In addn. immunofluorescence with confocal microscopy demonstrated lumenal staining of nasal polyp endothelium indicating that P-selectin was located on the surface of endothelial cells while in skin only an intracellular granular distribution was apparent. Lastly, whereas eosinophils **bound** consistently to nasal polyp endothelium, no **binding** was obsd. to blood vessels in normal skin further supporting the idea that eosinophils were **binding** to membrane expressed and not intracellular P-selectin. The importance of P-selectin in eosinophil adhesion to nasal polyp endothelium suggests that P-selectin antagonists may be effective at inhibiting eosinophil accumulation at sites of allergic inflammation.

L122 ANSWER 2 OF 28 CA COPYRIGHT 1996 ACS DUPLICATE 2
AN 119:26594 CA
TI Heterologous desensitization of the high affinity receptor for IgE (Fc.epsilon.R1) on RBL cells
SO J. Immunol. (1993), 150(9), 4072-83
CODEN: JOIMA3; ISSN: 0022-1767
AU Weetall, Marla; Holowka, David; Baird, Barbara
PY 1993
AB To study the properties of **Fc**.epsilon.RI desensitization induced by aggregation of that receptor, RBL cells were sensitized with a mixt. of two different IgE mAb to create two different populations of IgE-receptor complexes. Crosslinking of one receptor population contg. anti-dinitrophenol (DNP) IgE with a **bivalent antigen** (Ag), 1-DNP-amino-12-biotinamidododecane)2-avidin ((DNP)2-avidin), results in desensitization of a subsequent response, both of the same receptor population (homologous desensitization) and of the second receptor population contg. anti-5-dimethylaminonaphthalene-1-sulfonyl (dansyl) IgE (heterologous desensitization). The extent of heterologous desensitization is dependent on several parameters, including the concn. of both the first and the second Ag and the densities of the resp. IgE populations. Heterologous desensitization of the Ca2+ response is more sensitive to the concn. of the second stimulus (dansyl-BSA) than heterologous desensitization of the degranulation response. AlF4-, which activated GTP-**binding** proteins, can effectively replace (DNP)2-avidin as the initial stimulant and desensitizing agent. Other agents that mobilize intracellular Ca2+ including thrombin and a Ca2+ ionophore are less effective at replacing (DNP)2-avidin. Because pre-stimulation with Ag does not desensitize subsequent responses to AlF4- or Ca2+ ionophore, it appears that signal transduction via **Fc**.epsilon.RI is impaired at an early step. Addn. of monovalent DNP hapten within .apprx.10 min after crosslinking by (DNP)2-avidin completely prevents the desensitization of the subsequent Ca2+ or degranulation response to dansyl-BSA. After longer times of incubation with DNP Ag, the DNP hapten becomes increasingly less effective at preventing the desensitization of the dansyl-BSA response, even though ongoing

=> dup rem l120
FILE 'CA' ENTERED AT 16:00:10 ON 02 APR 96
USE IS SUBJECT TO THE TERMS OF YOUR CUSTOMER AGREEMENT
COPYRIGHT (C) 1996 AMERICAN CHEMICAL SOCIETY (ACS)

FILE 'CAPLUS' ENTERED AT 16:00:10 ON 02 APR 96
USE IS SUBJECT TO THE TERMS OF YOUR CUSTOMER AGREEMENT
COPYRIGHT (C) 1996 AMERICAN CHEMICAL SOCIETY (ACS)
PROCESSING COMPLETED FOR L120
L122 28 DUP REM L120 (28 DUPLICATES REMOVED)

=> d 1-28 .beverly; fil
biosi,medl,embas,lifesci,biotechds,cancerlit,wpids,confsi,dissabs,scisearch

L122 ANSWER 1 OF 28 CA COPYRIGHT 1996 ACS DUPLICATE 1
AN 121:33034 CA
TI Eosinophil adhesion to nasal polyp endothelium is P-selectin-dependent
SO J. Exp. Med. (1994), 180(1), 371-6
CODEN: JEMEAV; ISSN: 0022-1007
AU Symon, Fiona A.; Walsh, Garry M.; Watson, Susan R.; Wardlaw, Andrew J.
PY 1994
AB Tissue eosinophilia is a characteristic feature of a no. of inflammatory diseases including asthma and nasal polyposis. Eosinophil migration into tissues is controlled in part by interactions between eosinophil adhesion receptors and counter-structures on the vascular endothelium. To det. the receptors used by eosinophils to adhere to vascular endothelium in allergic inflammation the authors have adapted the Stamper-Woodruff frozen section assay (FSA) to study eosinophil adhesion to nasal polyp endothelium. Immunohistol. indicated that intercellular adhesion mol. 1 (ICAM-1), E-selectin, and P-selectin were well expressed by nasal polyp endothelium, whereas expression of vascular cell adhesion mol. 1 (VCAM-1) was weak or absent. Unstimulated human peripheral blood eosinophils adhered specifically to nasal polyp endothelium. Adherence was temp. and **divalent** cation-dependent and saturable at cell densities >5.times.106 cells/mL. Eosinophil adhesion was almost completely inhibited by a monoclonal antibody (mAb) against P-selectin and by a chimeric mol. consisting of the **Fc** portion of human IgG and the lectin **binding** domain of P-selectin, which **binds** to the P-selectin ligand on leukocytes. Anti-Mac-1 mAb partially inhibited eosinophil adhesion whereas mAb against E-selectin, L-selectin, ICAM-1, VCAM-1, very late activation **antigen** 4, and lymphocyte function-assocd. **antigen** 1 had no effect. P-selectin is stored in intracellular granules within the endothelial cell and in vitro is also transiently expressed. To det. if P-selectin was expressed on the membrane of the nasal polyp endothelium the authors compared P-selectin expression in normal skin and nasal polyps after acetone fixation, which permeabilizes cells, and paraformaldehyde, which only allows staining of membrane expressed receptors. In the skin, good expression was seen with

L122 ANSWER 6 OF 28 CA COPYRIGHT 1996 ACS DUPLICATE 6
AN 117:22534 CA
TI **Binding** parameters and idiotypic profile of the whole immunoglobulin and Fab' fragments of murine monoclonal antibody to distinct determinants of the human high molecular weight-melanoma associated antigen
SO Cancer Res. (1992), 52(9), 2497-503
CODEN: CNREA8; ISSN: 0008-5472
AU Temponi, Massimo; Gold, Allen M.; Ferrone, Soldano
PY 1992
AB The nonspecific accumulation of radioactivity in bone marrow, liver, and spleen in patients with melanoma injected with radiolabeled whole IgG of anti-high mol. wt.-melanoma assocd. **antigen** (HMW-MAA) monoclonal antibody (mAb) hampers the application of immunoscintigraphy to visualize melanoma lesions. This nonspecific background can be reduced by utilizing fragments which do not contain the **Fc** portion of anti-HMW-MAA mAb. Since the in vivo targeting to melanoma lesions of radiolabeled F(ab')2 and Fab' fragments of anti-HMW-MAA mAb is critically dependent on their **binding** parameters, the effect of fragmentation was studied on the **binding** characteristics of the anti-HMW-MAA mAbs 225.28, 763.74, and TP41.2. The 3 mAbs recognize distinct and spatially distant determinants with a heterogeneous distribution on the pool of HMW-MAA mols. synthesized by melanoma cells. The determinant recognized by mAb TP41.2 is detectable on a markedly smaller population of HMW-MAA mols. than those recognized by mAbs 225.28 and 763.74. 125I-labeled F(ab')2 fragments of the 3 mAbs displayed an immunoreactive fraction similar to that of the whole IgG, while Fab' fragments displayed a lower one. Fragmentation of mAbs 22.528 and TP41.2 to F(ab')2 produced a 2- and 1.5-fold redn. in their assocn. consts. but did not cause a significant change in that of mAb 763.74. Cleavage of F(ab')2 fragments to Fab' fragments produced 2-, 40-, and 7-fold redns. in the assocn. consts. of mAb 225.28, 763.74, and TP41.2, resp. These changes qual. fit the predictions of theory for univalent and **bivalent** mAb **binding**, since mAb 763.74 and TP41.2 appear to show **bivalent binding** to melanoma cells and mAb 225.28 to show univalent **binding**. The affinity const. of IgG, F(ab')2, and Fab' fragments of mAb 225.28, 763.74, and TP41.2 displays an inverse relation with the extent of their time-dependent release from the membrane of melanoma cells. Since no endocytosis of mAb was detected, the latter results suggest that radioactivity remains **bound** to melanoma cells in vivo for a longer time following injection of F(ab')2 fragments than following that of Fab' fragments of each of the anti-HMW-MAA mAb tested. Radiolabeled Fab' fragments of mAb 763.74 and TP41.2 displayed a marked redn. in their reactivity with some of the antiidiotypic mAb tested. The loss of some idiotopes is likely to be caused by changes in the conformation of the mols. assocd. with the fragmentation of IgG and by damage during the iodination procedure.

L122 ANSWER 7 OF 28 CA COPYRIGHT 1996 ACS DUPLICATE 7

AN 112:215045 CA
TI An alternative leukocyte homotypic adhesion mechanism, LFA-1/ICAM-1-independent, triggered through the human VLA-4 integrin
SO J. Cell Biol. (1990), 110(6), 2157-65
CODEN: JCLBA3; ISSN: 0021-9525
AU Campanero, M. R.; Pulido, R.; Ursa, M. A.; Rodriguez-Moya, M.; De Landazuri, M. O.; Sanchez-Madrid, F.
PY 1990
AB The VLA-4 (CD49d/CD29) integrin is the only member of the VLA family expressed by resting lymphoid cells that has been involved in cell-cell adhesive interactions. Here is described the triggering of homotypic cell aggregation of peripheral blood T lymphocytes and myelomonocytic cells by mAbs specific for certain epitopes of the human VLA .alpha.4 subunit. This anti-VLA-4-induced cell adhesion is isotype and **Fc** independent. Similar to phorbol ester-induced homotypic adhesion, cell aggregation triggered through VLA-4 requires the presence of **divalent** cations, integrity of cytoskeleton and active metab. Both adhesion phenomena differed in their kinetics and temp. requirements. This is an LFA-1/ICAM-1-independent process. The ability to inhibit this aggregation by other anti-VLA-4-specific antibodies recognizing epitopes on either the VLA .alpha.4 (CD49d) or .beta. (CD29) chains suggests that VLA-4 is directly involved in the adhesion process. The simultaneous **binding** of a pair of aggregation-inducing mAbs specific for distinct **antigenic** sites on the .alpha.4 chain resulted in the abrogation of cell aggregation. Thus, VLA-4-mediated aggregation may constitute a novel leukocyte adhesion pathway.

L122 ANSWER 8 OF 28 CA COPYRIGHT 1996 ACS DUPLICATE 8
AN 113:130198 CA
TI Site-directed immobilization of proteins
SO J. Chromatogr. (1990), 510, 293-302
CODEN: JOCRAM; ISSN: 0021-9673
AU Domen, Patricia L.; Nevens, Jeffrey R.; Mallia, A. Krishna; Hermanson, Greg T.; Klenk, Dennis C.
PY 1990
AB To det. if immobilization chem. can be used to orient antibody on a support so that the **bivalent binding** potential can be fully utilized, three activated matrixes were developed that couple to different functional groups on the mol. When AminoLink Gel was used to couple antibody randomly through primary amino groups, the molar ratio of immobilized antibody to recovered **antigen** averaged 1:1. Iodoacetyl groups on SulfoLink Gel couple through sulfhydryls in the hinge region of the antibody mol., in theory leaving the **antigen binding** site available. However, the antibody-to-**antigen** molar ratio was only slightly improved. Hydrazide groups on CarboLink Gel couple to aldehyde groups generated by oxidn. of carbohydrate moities that are located primarily on the **Fc** portion of the antibody mol. The molar ratio of immobilized antibody to purified **antigen** using CarboLink Gel reached the optimum of 1:2. CarboLink Gel is most effective at orienting antibody for

better **antigen** purifn. capability.

L122 ANSWER 9 OF 28 CA COPYRIGHT 1996 ACS DUPLICATE 9
AN 112:194907 CA
TI Proteins **bound** to a marker or solid-phase support matrix using a hydrazone linkage
SO U.S., 7 pp.
CODEN: USXXAM
IN O'Shannessy, Daniel J.
AI US 87-12456 870209
PI US 4874813 A 891017
PY 1989
AB A biol. compn. SPR1R2C(:O)NHN:CHP (SP = solid-phase support matrix or marker mol.; R1 = linking group; R2 = spacer arm having a chain of .gtoreq.6 atoms, with .gtoreq.1 atom being a tertiary amine which is protonated at pH <8, the remaining atoms being C, O, and/or N; P = protein) is provided. The hydrazone is formed by reacting SP or a marker mol., having R1 and R2 including a terminal hydrazide group, with a glycoprotein oxidized to include a terminal aldehyde group. Immunol. reagents prepd. by linking an Ig (IgG) through a carbohydrate on the **Fc** or hinge regions provide very high **binding** capacity approaching the theor. **bivalent** limit of 2 mol **bound antigen**/mol **bound** Ig. Thus, Affi-Gel 15 was reacted with hydrazine hydrate, and an oxidized monoclonal antibody to tissue plasminogen activator (tPA) was covalently coupled to the Affi-Gel 15 hydrazide. The product was able to **bind** and release tPA **antigen** at a **binding** efficiency of .apprx.1.81 mol **antigen**/mol antibody, which is 90.7% of the theor. native antibody **binding** capacity.

L122 ANSWER 10 OF 28 CA COPYRIGHT 1996 ACS DUPLICATE 10
AN 111:113388 CA
TI Tryptic digestion of Xenopus IgM and IgY molecules
SO Dev. Comp. Immunol. (1989), 13(2), 149-57
CODEN: DCIMDQ; ISSN: 0145-305X
AU Coosemans, Veronique; Hadji-Azimi, Irandokht; Perrenot, Nadine
PY 1989
AB Xenopus IgM and IgY mols. were digested by trypsin. Their resp. fragments were sepd. by gel filtration and immunoadsorption. The purified fragments were characterized by SDS-PAGE and immunoblotting. Tryptic digestion of Xenopus IgM resulted in the release, at a low yield, of hexameric **Fc**.mu., and of monovalent Fab.mu. fragments. The digestion of Xenopus IgY antibodies led to the recovery of **divalent** and monovalent Fab .upsilon. fragments. The **antigen-binding** property of these fragments was demonstrated. No **Fc** .upsilon. fragments of appreciable size could be detected.

L122 ANSWER 11 OF 28 CA COPYRIGHT 1996 ACS DUPLICATE 11
AN 112:116934 CA
TI Attack on neoplastic cell membranes by therapeutic antibody
SO Mol. Cell. Biochem. (1989), 91(1-2), 33-8

CODEN: MCBIB8; ISSN: 0300-8177
AU Stevenson, G. T.
PY 1989
AB Mouse monoclonal antibody is not well fitted to destroying tumor cell targets. Complement and cellular effectors are inefficiently recruited, the cells can undergo **antigenic** modulation, **antigen**-neg. mutants can arise, and the tumor-bearing subject can mount an immune response against the therapeutic antibody. This paper describes the prepn. of 2 chimeric antibody derivs. designed to circumvent some of these problems. The first deriv. is FabFc, prepd. by linking Fab'.gamma. from monoclonal antibody to **Fc**.gamma. from human IgG. The bismaleimide linking agent forms a thioether bond with an SH group released by redn. of SS bonds in the hinge of each constituent. The second deriv. is bisFabFc, formed by a bismaleimide in this case joining 2 FabFc mols. via a free SH in the **Fc** hinge of each. As regards antibody activity against target cells bisFabFc can be univalent (one active, one inactive Fab arm), **bivalent**, or bispecific (with each Fab arm directed against a different cell surface **antigen**). Its juxtaposed dual **Fc** regions are designed to promote cooperative **binding** of effectors. Some preliminary characterization in vitro has employed antibodies of anti-idiotypic specificity directed against guinea-pig L2C leukemic B lymphocytes. The parent mouse IgG1 antibody failed to invoke complement cytotoxicity or antibody-dependent cellular cytotoxicity, while the chimeric derivs. yielded good killing in both systems. In complement lysis **bivalent** bisFabFc outperformed univalent, which in turn outperformed the FabFc monomer.

L122 ANSWER 12 OF 28 CA COPYRIGHT 1996 ACS DUPLICATE 12
AN 105:59157 CA
TI Fc:Fc interactions revealed by spin-labeled IgG heterosaccharides in model immune complexes
SO Biochem. Biophys. Res. Commun. (1986), 137(1), 237-43
CODEN: BBRCA9; ISSN: 0006-291X
AU Kusumi, Akihiro; Winkelhake, Jeffrey L.
PY 1986
AB Dynamic properties of spin-labeled heterosaccharides in the **Fc**-region of murine monoclonal antihapten IgG were studied in model immune complexes (IC) as a function of the IC size. Model IC dimers, trimers and oligomers were formed using **bivalent** photoaffinity **antigens**. The ESR spectrum exhibits 2 components. The rotational correlation time of the less-immobilized species is shorter than 10-10 s, and that of the more-immobilized component is in the order to 10-9 -10-8 s depending on the IC size. The fraction of the more-immobilized spin labels increases, and the mobility of this component decreases with increase in IC size (i.e., mobility: monomers .apprx. dimers > trimers .mchgt. immune-complex ppts.). These data strongly suggest the existence of **Fc**: **Fc** interactions in IC, and provide the basis for a model in which such interactions underlie the initial mechanism by which the information of **antigen binding** to Fab region is

transferred into organized **Fc:Fc** assocn. structure for IgG effector activities.

L122 ANSWER 13 OF 28 CA COPYRIGHT 1996 ACS DUPLICATE 13
AN 103:69562 CA
TI Activation of complement pathways by univalent antibody derivatives with intact Fc zones
SO Mol. Immunol. (1985), 22(7), 803-10
CODEN: MOIMD5; ISSN: 0161-5890
AU Watts, H. F.; Anderson, V. A.; Cole, V. M.; Stevenson, G. T.
PY 1985
AB The ability of 2 univalent antibody derivs. to invoke complement-mediated lysis of guinea-pig L2C leukemic lymphocytes was investigated. The derivs. were Fab/c from rabbit IgG antibody, in which only one Fab arm is removed from the parent mol., and FabFc, in which Fab'.gamma., from peptic digestion of sheep IgG antibody, is disulfide-bonded to the **Fc**.gamma. obtained by papain digestion of an arbitrary IgG. Antibody activity was directed against surface IgM on the target cells. Both derivs. could invoke lysis via the classical pathway in the presence of rabbit complement. Exposure of the cells to the derivs. at 37.degree. before introducing complement yielded no protective **antigenic** modulation. At low complement concns. the derivs. were more efficient than the parent antibodies at invoking lysis, apparently due to the fact that the derivs. do not cause modulation; it appears that cells can undergo a useful degree of modulation when confronted simultaneously by **bivalent** antibody and low levels of complement. The Fab/c prepns. were also able to invoke lysis by guinea-pig complement. Lysis occurred under conditions where activation took place only via the classical pathway (in dil. complement) or only via the alternative pathway (in the absence of Ca ions, or in C4-deficient guinea-pig serum). Thus, there is no need for 2 **antigen-binding** Fab arms in antibody activation of either the classical or alternative complement pathways. They favor models requiring clustering of **Fc** regions rather than steric changes which might follow **binding** of **antigen**.

L122 ANSWER 14 OF 28 CA COPYRIGHT 1996 ACS DUPLICATE 14
AN 102:147362 CA
TI A lectinlike receptor on murine macrophage cell line cells, Mm1: involvement of sialic acid-**binding** sites in opsonin-independent phagocytosis for xenogeneic red cells
SO J. Leukocyte Biol. (1985), 37(3), 289-304
CODEN: JLBIE7; ISSN: 0741-5400
AU Kyoizumi, Seishi; Masuda, Tohru
PY 1985
AB The recognition mechanism of xenogeneic red cells by mouse macrophages was studied by using established cell lines. Approx. 30% of cell line cells Mm1 which lack Ia **antigen**, as well as of thioglycollate-induced peritoneal macrophages from SL/Am mice (TGC-M.vphi.) could ingest unopsonized quail red cells (QRC). In contrast, an undifferentiated type of cell line, M1-, and another

type of macrophage cell line, Mk1-C, possessing accessory cell activity in assocn. with the expression of Ia **antigen**, had no phagocytic activity for QRC. Approx. 80% of Mm1 cells, as well as TGC-M.vphi. formed rosettes with QRC, whereas M1- and Mk1-C cells did not; indicating that specific **binding** sites for QRC are expressed on a large portion of Mm1 and TGC-M.vphi. but not on M1- and Mk1-C cells. No requirement of **divalent** cation (Mg2+, Ca2+) and metabolic energy was obsd. for rosette formation between Mm1 cells and QRC. Protease treatment of Mm1 cells eliminated the rosetting activity, whereas periodate oxidn. of glycosidase treatment slightly enhanced this activity, suggesting the involvement of surface protein in **binding** sites of Mm1 cells. In contrast to these findings on Mm1 cells, **binding** components of QRC were sensitive to periodate oxidn. or neuraminidase treatment but resistant to protease, suggesting that the terminal sialic acid residues of carbohydrate of QRC are recognized by Mm1 cells. Furthermore, N-acetylneuraminic acid (NeuNAc) inhibited the rosette formation and promoted the dissocn. of rosettes already formed. N-acetylneuramin lactose was more efficient in rosette inhibition than NeuNAc. These sugars also blocked the phagocytosis of QRC by Mm1 cells but had no effect on either **Fc**-mediated phagocytosis or latex ingestion. These results suggest that phagocytosis of QRC by murine macrophages is mediated by protease-sensitive **binding** sites recognizing terminal sialic acid residues of QRC in conjunction with addnl. carbohydrates.

L122 ANSWER 15 OF 28 CA COPYRIGHT 1996 ACS DUPLICATE 15
AN 101:189437 CA
TI The **binding** of monoclonal antibodies to cell surface molecules. Quantitative analysis of the reactions and cross-reactions of an antibody (MB40.3) with four HLA-B molecules
SO J. Biol. Chem. (1984), 259(21), 13077-83
CODEN: JBCHA3; ISSN: 0021-9258
AU Parham, Peter
PY 1984
AB The MB40.3 monoclonal antibody **binds** to 4 distinct HLA-B **antigen** mols.; B7, B40, B40*, and B27. With Fab' fragments only the interaction with B7 and B40 was detected and the affinity for both was the same (1-2 .times. 108M-1), suggesting the epitope is shared by the 2 mols. Unlike many antibodies for which low affinity is due to a high-dissocn. const., that of MB40.3 results from a very low-assocn. rate const., coupled with a low-dissocn. const. In consequence, the affinity and avidity of Fab', F(ab')2, and IgG for B7 and B40 were of similar magnitude, sol. B7 was a more efficient competitor for antibody than cell surface B7, and in practice antibody **bivalency** was of little importance. The forward rate const. could be increased by removing **Fc** from the antibody or by removing sialic acid from the cells by treatment with neuraminidase. The neuraminidase treatment also produced an increase in the no. of detectable cell surface HLA-A,B mols. The affinity of MB40.3 for B40* and B27 was estd. to be <4 .times. 106 as no **binding** with Fab' was detected due to a

high-dissocn. rate. For these 2 HLA-B mols. **bivalent** attachment was crit., and it increased the strength of interaction with cell surface B40* and B27 to a point where the avidities were comparable to those obtained with B7 and B40, with B40* interacting more strongly than B27. The epitopes recognized by MB40.3 on B40* and B27 were thus shown to be structurally different from each other and from those on B7 and B40.

L122 ANSWER 16 OF 28 CA COPYRIGHT 1996 ACS DUPLICATE 16
AN 102:22533 CA
TI Regulation of T-cell function by antibodies: Enhancement of the response of human T-cell clones to hepatitis B surface antigen by antigen-specific monoclonal antibodies
SO Proc. Natl. Acad. Sci. U. S. A. (1984), 81(21), 6846-50
CODEN: PNASA6; ISSN: 0027-8424
AU Celis, Esteban; Zurawski, Vincent R., Jr.; Chang, Tse Wen
PY 1984
AB Eight mouse monoclonal antibodies specific for hepatitis B surface **antigen** (HBsAg) were examd. for their effects on the **antigen**-induced proliferative response and lymphokine prodn. of human HBs Ag-specific T-cell clones in vitro. Although all Igs specifically enhanced the T-cell proliferative response, antibodies of the IgG class were generally more effective than those of the IgM class. Both the **divalent** F(ab')2 and the monovalent Fab fragments of an IgG monoclonal antibody had no effects, indicating that the **Fc** portion of the antibody mols. was required. Since **antigen**-presenting cells bear surface receptors for the **Fc** of IgG and fewer or none for that of IgM, the results also suggest that antibodies enhance the capture of **antigens** by **antigen**-presenting cells as a result of the **binding** of **antigen**-antibody complexes to the **Fc** receptors on these cells. In addn. to potentiating the proliferation of the T-cell clones, antibodies also increased the **antigen**-induced prodn. of interferon-.gamma. by these cells. Antibodies may regulate immune responses and do so by enhancing **antigen** presentation, thus augmenting **antigen**-induced activation and clonal expansion of T cells.

L122 ANSWER 17 OF 28 CA COPYRIGHT 1996 ACS DUPLICATE 17
AN 99:210930 CA
TI Suppression of in vitro monoclonal human rheumatoid factor synthesis by antiidiotypic antibody. Target cells and molecular requirements
SO J. Clin. Invest. (1983), 72(4), 1410-19
CODEN: JCINAO; ISSN: 0021-9738
AU Koopman, William J.; Schrohenloher, Ralph E.; Barton, James C.; Greenleaf, Edward C.
PY 1983
AB Previous studies have indicated that anti-idiotypic antibody can modulate expression of idiotype both in vivo and in vitro. Although the precise mechanisms underlying modulation of idiotype expression by anti-idiotype antibody remains unclear, a requirement for intact IgG anti-idiotypic antibody has been suggested and T cells appear to play a role in some systems. Peripheral blood mononuclear

leukocytes (MNL) from a patient with a B cell lymphoma and a circulating IgM.kappa. rheumatoid factor (RF) paraprotein were studied in an effort to delineate mechanisms involved in the regulation of idiotype expression by anti-idiotypic antibody. One-10% of MNL from this patient could be cytoplasmically stained with specific F (ab')2 anti-idiotypic antibody. MNL from the patient spontaneously synthesized IgM RF in culture that possessed the same idiotype as the circulating IgM RF paraprotein. Prodn. of RF by MNL was suppressed by pretreatment with either intact IgG or the F(ab')2 fragments of anti-idiotypic antibody. In contrast, the Fab' fragment of anti-idiotypic antibody was not inhibitory despite retaining demonstrable anti-idiotype activity. Suppression of RF prodn. was not obsd. over the same concn. range with the IgG or F(ab')2 fractions of a noncross-reactive anti-idiotypic antibody prepd. against another monoclonal IgM.kappa. RF paraportein or with IgG or F(ab')2 fractions prepd. from normal rabbit serum. Inhibition of RF prodn. by anti-idiotypic antibody did not require T cells. Anti-idiotypic antibody decreased intracellular and extracellular levels of idiotype indicating diminished synthesis of idiotype by the patient's B cells. Synthesis of IgM RF by MNL obtained from unrelated donors was not suppressed by the anti-idiotypic antibody specific for the patient's paraprotein. Apparently, (a) anti-idiotypic antibody is capable of directly suppressing human B cell release of idiotype, (b) the **bivalent antigen-binding** fragment [F(ab')2] of anti-idiotypic antibody is sufficient for mediating such suppression, (c) an intact **Fc** portion of anti-idiotypic antibody enhances suppression of idiotype, and (d) anti-idiotypic antibody inhibits idiotype expression by suppressing synthesis of idiotype.

L122 ANSWER 18 OF 28 CA COPYRIGHT 1996 ACS DUPLICATE 18
AN 99:210949 CA
TI The **binding** of monoclonal antibodies to cell-surface molecules. A quantitative analysis with immunoglobulin G against two alloantigenic determinants of the human transplantation antigen HLA-A2
SO Biochem. J. (1983), 216(2), 423-32
CODEN: BIJOAK; ISSN: 0306-3275
AU Ways, Judy P.; Parham, Peter
PY 1983
AB Monoclonal IgG1 PA2.1 and MA2.1 antibodies recognize polymorphic sites of the human transplantation **antigen** HLA-A2. They are distinguishable because Ma2.1 **binds** HLA-A2 and HLA-B17, whereas PA2.1 **binds** HLA-A2 and HLA-A28*. The affinities of PA2.1-Fab for HLA-A2, 3 HLA-A2 variants, and HLA-A28* are similar and relatively low (1.9 .times. 107 M-1). The affinities of MA2.1-Fab for HLA-A2, 3 HLA-A2 variants, and HLA-B17 are similar and high (1.2 .times. 109 M-1). The difference in affinity is due to the rates of dissocn., which give half-times of dissocn. of 290 min for MA2.1-Fab and 4 min for PA2.1-Fab. For both Fab, equil. measurements and kinetic detns. gave consistent ests. for affinity. When PA2.1-F(ab)2 or IgG is incubated with cells, it

reaches equil. within 3 h, with most mols. **bound** **bivalently** to the cell. Under similar conditions, MA2.1-F(ab)2 does not reach equil. and a significant proportion of mols. **bound** with 1 and 2 sites are found. For the lower-affinity antibody (PA2.1), ests. of the **binding** consts. for 1- and 2-site interactions could be made. By simple Scatchard anal., the avidity of F(ab)2 or IgG is 1.3 .times. 109 M-1, giving an enhancement factor of 68 between **bivalent** and univalent **binding**. This is a measure of the equil. const. for the interchange between **bivalent** and univalent **binding**. Anal. of the results with more realistic models indicates that the actual value is larger (103-104 M-1) than 68 M-1. The avidities of F(ab)2 and IgG for HLA-A2 are identical, showing the **Fc** does not interfere with **bivalent** **binding** to cells.

L122 ANSWER 19 OF 28 CA COPYRIGHT 1996 ACS DUPLICATE 19
AN 98:213869 CA
TI Properties of two monoclonal antibodies directed against the Fc and Fab regions of rat IgE
SO Int. Arch. Allergy Appl. Immunol. (1983), 70(4), 352-60
CODEN: IAAAAM; ISSN: 0020-5915
AU Conrad, Daniel H.; Studer, Elaine; Gervasoni, Jim; Mohanakumar, T.
PY 1983
AB Hybridoma antibodes directed against the **Fc** and Fab portions of rat IgE were produced by immunizing BALB/c mice with rat IgE and fusing the spleen cells with the nonsecreting plasmacytoma P3/X63Ag8.653. Two of the antibodies, designated as A2 and B5, were extensively characterized. Competitive **binding** expts. using rat IgE from the IR 162 and IR2 immunocytomas and rat IgG indicated that both A2 and B5 were .epsilon.-chain specific and not anti-idiotype. A2 also exhibited some cross-reactivity with mouse IgE. When IgE was treated with chymotrypsin so as to produce both F(ab')2 and Fab fragments, the enzyme-treated IgE retained reactivity with B5, but the reactivity to A2 was lost. Heat denaturation of IgE at 56.degree. resulted in a progressive loss of reactivity of the IgE for both A2 and the **Fc** receptor on rat basophilic leukemia cells; the reactivity of B5 remained unchanged. A2 does not interact with the same site on the **Fc** of IgE that is involved in **binding** to the rat basophilic leukemia cell **Fc** receptor; A2 exerted little influence on the **binding** of IgE to rat basophilic leukemia cells. Thus, the **antigenic** site for B5 is in the Fab region of the IgE mol. and A2 reacts with the IgE **Fc**. Use of these antibodies to measure cell-**bound** IgE was also evaluated in dual label expts., and potential problems in using **divalent** antibodies to quantitate cell surface **antigens** are discussed.

L122 ANSWER 20 OF 28 CA COPYRIGHT 1996 ACS DUPLICATE 20
AN 96:66980 CA
TI The effect of IgG and IgG fragments on the absorption of inhaled antigens across the air-blood barrier of isolated perfused rabbit

lungs
SO Immunology (1981), 44(2), 331-7
CODEN: IMMUAM; ISSN: 0019-2805
AU Geoghegan, W. D.; Dawson, C. A.; Calvanico, N. J.
PY 1981
AB Whole antiserum and the IgG fraction of whole antiserum were equally effective in inhibiting the **antigen** absorption of inhaled sol. **antigens** across the alveolocapillary membrane. The F(ab')2 and Fab' fragments of IgG were as effective as native IgG. The **antigen-binding** site of IgG is, therefore, the only structural requirement, with the **Fc** receptor and **divalent antigen binding** not being required.

L122 ANSWER 21 OF 28 CA COPYRIGHT 1996 ACS DUPLICATE 21
AN 91:3680 CA
TI Interaction of a bivalent ligand with IgM anti-lactose antibody
SO Biochemistry (1979), 18(11), 2226-32
CODEN: BICHAW; ISSN: 0006-2960
AU Karush, Fred; Chua, Ming-Ming; Rodwell, John D.
PY 1979
AB A model system was developed for the study of the interaction between **bivalent** ligands and multivalent antibody. This system utilized modified .beta.2-microglobulin as the carrier of the reactive lactosyl groups and equine antilactose IgM antibody. Chem. modification of the carrier allowed conjugation of 2 such groups to cysteinyl residues at positions 25 and 81 with a potential sepn. of 20 nm. Each lactosyl group contained a 2,4-dinitrophenyl moiety which served as a sensor of the **binding** measurements by fluorescence quenching. Examn. by anal. ultracentrifugation demonstrated no significant cross-linking by the **bivalent** ligand of either the IgM antibody or the monomer (IgMs) derived from it. **Binding** consts. for complex formation between the **bivalent** and monovalent ligands and IgM, IgMs, and Fab.mu. were detd. by fluorescence titrns. These measurements also established that the **bivalent** ligand was **bound** to IgM and IgMs primarily as a cyclic complex. The small enhancement factor for the **binding** of the **bivalent** ligand compared with the monovalent one was attributed to the loss of configurational entropy assocd. with the formation of the cyclic complex. The complex of **bivalent** ligand and IgM antibody was effective in the depletion of complement in contrast to the complex with the small monovalent ligand. Thus, this **antigen**-mediated effector activity is the consequence of a distortion of the normal planar structure of the IgM mol. The structural perturbation would consist of a bending of some of the F(ab')2 regions out of the plane contg. the (**Fc**.mu.)5 core due to the cross-linking of adjacent F(ab')2 regions by the **bivalent** ligand.

L122 ANSWER 22 OF 28 CA COPYRIGHT 1996 ACS DUPLICATE 22
AN 92:4567 CA
TI Degranulation of human basophils: quantitative analysis of

histamine release and desensitization, due to a bivalent penicilloyl hapten

SO J. Immunol. (1979), 123(4), 1864-72
CODEN: JOIMA3; ISSN: 0022-1767

AU Dembo, Micah; Goldstein, Byron; Sobotka, Anne K.; Lichtenstein, Lawrence M.

PY 1979

AB A quant. model of histamine release from human basophils due to the sym. **bivalent** hapten, bis(benzylpencilloyl)-1,6-diaminohexane, (BPO)2 is presented. The major elements of the model are: the histamine content of basophils is divided into a large no. of discrete and quasi-independent quanta; a certain fraction of the quanta in a population of basophils are nonreleasable; release of the contents of a quanta is a stochastic event; the **binding** and cross-linking reaction between (BPO)2 and cell surface IgE approaches thermal equil. on a time scale that is very short compared to the time scale of histamine release from or desensitization of basophils; the probability per unit time of the release event, PR, is a function of the extracellular Ca concn. [Ca]ex, and of the no. of cross-linked IgE mols. per cell, Xpoly; in addn., the functional dependence of PR or [Ca]ex is such that PR .fwdarw. 0 as [Ca]ex .fwdarw. 0 for all values of Xpoly; and **antigen**-specific IgE mols. and/or their assocd. **Fc** receptors are inactivated at a rate that is a saturable function of Xpoly; furthermore, this process of inactivation is not dependent on the extracellular Ca concn. The model was tested by carrying out a detailed fitting to histamine release data obtained by using cells from a single donor. Good agreement between theory and expt. was obtained, and all the parameters of the model were detd. The ways in which the model can be extended to include systems more complex than the (BPO)2 system are briefly discussed.

L122 ANSWER 23 OF 28 CA COPYRIGHT 1996 ACS DUPLICATE 23

AN 88:134691 CA

TI Mobile Fc region in the Zie IgG2 cryoglobulin: comparison of crystals of the F(ab')2 fragment and the intact immunoglobulin

SO Biochemistry (1978), 17(5), 820-3
CODEN: BICHAW; ISSN: 0006-2960

AU Ely, K. R.; Colman, P. M.; Abola, E. E.; Hess, A. C.; Peabody, D. S.; Parr, D. M.; Connell, G. E.; Laschinger, C. A.; Edmundson, A. B.

PY 1978

AB A human IgG2(.kappa.) (Zie) and the **bivalent** **antigen-binding** fragment, F(ab')2, derived from it by peptic hydrolysis were crystd. in forms suitable for x-ray diffraction studies. The IgG2 mol. crystd. at 15.degree. in 0.1M Na borate-0.05M NaCl, pH 7.0, and the F(ab')2 fragment crystd. in 0.02M Na phosphate, pH 7.4. The space group was I222 or I212121, with a = 100.3, b = 153.1, and c = 188.5 .ANG. for the IgG2 protein and a = 99.6, b = 150.7, and c = 192.7 .ANG. for the F(ab')2 fragment. The striking similarities indicate that the structural features contributing to the diffraction patterns are nearly the same in the 2 crystals. Thus, the Fab' regions have similar conformations in the fragment and parent mol. and the **Fc** region does not

contribute significantly to the diffraction pattern of the IgG2 protein. The ability of the **Fc** region to adopt >1 conformation with respect to the Fab' arms in the crystal lattice adds to previous chem. and crystallog. evidence for the biol. importance of the flexibility of Ig mols.

L122 ANSWER 24 OF 28 CA COPYRIGHT 1996 ACS DUPLICATE 24
AN 86:104362 CA
TI Recognition and allostery in the mechanism of antibody action
SO Colloq. Ges. Biol. Chem. (1976), 27(Immune Syst.), 41-54
CODEN: CGBCA9
AU Pecht, I.
PY 1976
AB Conformational transitions induced by hapten (contg. an o-dinitro arom. ring) **binding** were obsd. Ig (MOPC 460) was found to exist in 2 conformational states, both when free or hapten **bound**. The transition between the different states is induced by the hapten **binding**. Following the circular polarization of the intrinsic fluorescence of the antibodies, the induction of conformational changes upon **antigen binding** was transmitted to the **Fc** domains. The examn. of these spectroscopic changes was extended and correlated with the induction of complement **binding**. For antibodies of the IgG class a correlation was found only when **divalent** or polyvalent **antigens** were **bound**, whereas for IgM class antibodies of the same specificity, **binding** of the monovalent **antigenic** determinant was sufficient for both activation of complement and induction of the changes in circular polarization of luminescence (CPL). These observations support the idea that the interaction between the **antigen binding** site and the effector sites of antibodies have an allosteric nature. The significance of the requirement of **antigen divalency** for IgG may be an inherent part of this nature so as to accommodate an appropriate angular relation between the different domains of the Ig mol.

L122 ANSWER 25 OF 28 CA COPYRIGHT 1996 ACS DUPLICATE 25
AN 80:131564 CA
TI In vitro suppression of immunoglobulin allotype synthesis by Fab and F(ab')2 fragments of anti-allotype antibody
SO Cell. Immunol. (1974), 11(1), 367-76
CODEN: CLIMB8
AU Schuffler, Carol; Dray, Sheldon
PY 1974
AB The F (ab')2 and Fab fragments were prepd. from IgG contg. antiallotype antibody (Ab) specific for the .kappa. light chains (i.e., anti-b4) and were tested for their ability to suppress b4 allotype prodn. in vitro. Spleen cells from a b4b5 heterozygous rabbit were incubated for 24 hr with the F (ab')2 or Fab fragments and then washed and cultured for an addnl. 4 days. The tissue culture supernatant fluids were analyzed for the b4 and b5 allotypes by a radioimmunoassay. The results clearly indicated that both types of IgG fragments can suppress the prodn. of the b4 allotype.

Thus, neither the **Fc** fragment nor the **bivalency** of the intact Ab mol. is essential for suppressing activity. This makes it highly unlikely that the allotype suppression in vitro occurs as a result of cell death. When equiv. amts. of the IgG, F (ab')2, or Fab anti-b4 prepns. as detd. by their **antigen binding** capacity, were compared for their relative efficiencies in the induction of b4 allotype suppression, the IgG and F (ab')2 were equally efficient, while the Fab fragment was less efficient than the **bivalent** Ab prepns.

L122 ANSWER 26 OF 28 CA COPYRIGHT 1996 ACS DUPLICATE 26
AN 77:17805 CA
TI Receptor for Fc on mouse B-lymphocytes
SO J. Immunol. (1972), 108(5), 1319-27
CODEN: JOIMA3
AU Paraskevas, F.; Lee, S. T.; Orr, K. B.; Israels, L. G.
PY 1972
AB **Antigen**-antibody complexes derived from rabbit antibodies **bind** to mouse spleen lymphocytes. This **binding** was detected by the inhibition of reverse immune cytoadherence (RICA), a technique detecting surface .gamma.-globulin and suggests that these complexes **bind** to .gamma.-globulin carrying B-cells. Complement is not a requirement for **binding**. Complexes made from 5 S **bivalent** antibodies do not **bind** nor does 7 S antibody or normal .gamma.-globulin. Chem. produced microaggregates of ferritin show no **binding**. Mouse complexes made with mouse .gamma.G (7S.gamma.2a) antibodies also **bind** to mouse spleen cells. **Fc** fragments isolated by papain digestion of rabbit antibodies also inhibit RICA and **Fc** crystals can form around the mouse spleen lymphocytes. **Fc** from normal rabbit .gamma.-globulin is less inhibitory. These results are presented as evidence of the existence of a receptor, provisionally called the **Fc** receptor, on the surface of mouse spleen lymphocytes which are probably B-cells.

L122 ANSWER 27 OF 28 CA COPYRIGHT 1996 ACS DUPLICATE 27
AN 76:44585 CA
TI Antibody-mediated suppression of the immune response in vitro. IV. Effect of antibody fragments
SO J. Immunol. (1972), 108(1), 93-101
CODEN: JOIMA3
AU Feldmann, Marc; Diener, Erwin
PY 1972
AB The capacity of purified Fab' and F(ab')2 pepsin antibody fragments to inhibit the immune response to polymd. flagellin in vitro was investigated. Marked differences between the activities of these fragments were found. Only high doses of univalent Fab' were suppressive, whereas much smaller amts. of **divalent** F(ab')2 were active. Preincubation expts. were performed to det. whether these fragments were capable of potentiating the induction of tolerance in vitro. Only F(ab')2 fragments facilitated the induction of antibody-mediated tolerance; Fab' fragments, regardless

of dose, were inactive. These findings highlighted several important aspects of the mechanism of antibody-induced suppression. First, the difference in activity of Fab' and F(ab')2, despite the same **antigen-binding** capacity and affinity, demonstrated the existence of .gtoreq.2 different mechanisms of antibody-induced immune suppression in vitro. Second, the importance of **divalency** for the induction of tolerance srongly supports the **antigen** cross-linking hypothesis of antibody-mediated tolerance. Third, the **Fc** part of the IgG mol. is not involved in the mechanism of antibody-mediated tolerance in vitro.

L122 ANSWER 28 OF 28 CA COPYRIGHT 1996 ACS DUPLICATE 28
AN 73:23499 CA
TI Significance of immunoglobulin E in reaginic hypersensitivity
SO Ann. Allergy (1970), 28(5), 189-202
CODEN: ANAEA3
AU Ishizaka, Kimishige; Ishizaka, Teruko
PY 1970
AB Human reaginic antibodies in sera of atopic patients were immunoglobulin E, a .gamma.1-glycoprotein with mol. wt. of 200,000. Immunoglobulin E was synthesized in plasma cells predominant in the respiratory and gastrointestinal tract. Immunochem. studies of reaginic antibodies indicate that the antibodies are **bivalent**, and sensitize human leukocytes and monkey lung tissues, but not guinea pig skin. The **Fc** fragments of .gamma.E antibodies passively sensitized human leukocytes and monkey lung tissues for reversed type reactions, indicating that .gamma.E combines with target cells through the **Fc** portion of the mol. .gamma.E-Globulin was present on basophil leukocytes but not on granulocytes or small lymphocytes. The mechanism for the reaginic hypersensitivity reaction proposed a bridging of a cell-**bound** .gamma.E mols. by **antigen**, resulting in interaction between the .gamma.E mols.; such interaction or consequent structural changes in the **Fc** portion of the .gamma.E mols. may induce enzymic sequences leading to the release of histamine and slow reactive substance-anaphylaxis (SRS-A) depending on the cells involved.

'CONFSI' IS NOT A VALID FILE NAME
Enter "HELP FILE NAMES" at an arrow prompt (=>) for a list of files that are available. If you have requested multiple files, you can specify a corrected file name or you can enter "IGNORE" to continue accessing the remaining file names entered.
ENTER A FILE NAME OR (IGNORE):end

=> fil
biosi,medl,embas,lifesci,biotechds,cancerlit,wpids,confsci,dissabs,scisearch
FILE 'BIOSIS' ENTERED AT 16:02:11 ON 02 APR 96
COPYRIGHT (C) 1996 BIOSIS(R)

```
FILE 'MEDLINE' ENTERED AT 16:02:11 ON 02 APR 96

FILE 'EMBASE' ENTERED AT 16:02:11 ON 02 APR 96
COPYRIGHT (C) 1996 Elsevier Science B.V.  All rights reserved.

FILE 'LIFESCI' ENTERED AT 16:02:11 ON 02 APR 96
COPYRIGHT (C) 1996 Cambridge Scientific Abstracts (CSA)


FILE 'BIOTECHDS' ENTERED AT 16:02:11 ON 02 APR 96
COPYRIGHT (C) 1996 DERWENT INFORMATION LTD

FILE 'CANCERLIT' ENTERED AT 16:02:11 ON 02 APR 96

FILE 'WPIDS' ENTERED AT 16:02:11 ON 02 APR 96
COPYRIGHT (C) 1996 DERWENT INFORMATION LTD

FILE 'CONFSCI' ENTERED AT 16:02:11 ON 02 APR 96
COPYRIGHT (C) 1996 Cambridge Scientific Abstracts (CSA)

FILE 'DISSABS' ENTERED AT 16:02:11 ON 02 APR 96
COPYRIGHT (C) 1996 University Microfilms, Inc.

FILE 'SCISEARCH' ENTERED AT 16:02:11 ON 02 APR 96
COPYRIGHT (C) 1996 Institute for Scientific Information (ISI) (R)

=> s (divalen? or di valen? or bivalen? or bi valen?)(l)(fc? or cd16 or cd
16)
L123       177 FILE BIOSIS
L124       160 FILE MEDLINE
L125       163 FILE EMBASE
L126        50 FILE LIFESCI
L127         4 FILE BIOTECHDS
L128        58 FILE CANCERLIT
L129        31 FILE WPIDS
L130         1 FILE CONFSCI
L131        20 FILE DISSABS
L132        67 FILE SCISEARCH

TOTAL FOR ALL FILES
L133       731 (DIVALEN? OR DI VALEN? OR BIVALEN? OR BI VALEN?)(L)(FC? OR
               CD16 OR CD 16)

=> s l133(l)antigen?
L134        44 FILE BIOSIS
L135        44 FILE MEDLINE
L136        51 FILE EMBASE
L137        13 FILE LIFESCI
L138         3 FILE BIOTECHDS
L139        25 FILE CANCERLIT
L140         8 FILE WPIDS
L141         0 FILE CONFSCI
L142         9 FILE DISSABS
```

```
L143          13 FILE SCISEARCH

TOTAL FOR ALL FILES
L144         210 L133(L) ANTIGEN?

=> s l144 not (l55 or l101)
L145          42 FILE BIOSIS
L146          42 FILE MEDLINE
L147          48 FILE EMBASE
L148          12 FILE LIFESCI
L149           2 FILE BIOTECHDS
L150          21 FILE CANCERLIT
L151           8 FILE WPIDS
L152           0 FILE CONFSCI
L153           6 FILE DISSABS
L154          12 FILE SCISEARCH

TOTAL FOR ALL FILES
L155         193 L144 NOT (L55 OR L101)

=> s ran=,1994
ENTER LOGIC EXPRESSION, QUERY NAME, OR (END):l155
L156          41 FILE BIOSIS
L157          40 FILE MEDLINE
L158          46 FILE EMBASE
L159          11 FILE LIFESCI
L160           1 FILE BIOTECHDS
L161          19 FILE CANCERLIT
L162           7 FILE WPIDS
L163           0 FILE CONFSCI
L164           4 FILE DISSABS
L165          11 FILE SCISEARCH

TOTAL FOR ALL FILES
L166         180 L155

=> s l166 and (bind? or bound)
L167          25 FILE BIOSIS
L168          25 FILE MEDLINE
L169          30 FILE EMBASE
L170           9 FILE LIFESCI
L171           0 FILE BIOTECHDS
L172          13 FILE CANCERLIT
L173           6 FILE WPIDS
L174           0 FILE CONFSCI
L175           3 FILE DISSABS
L176           4 FILE SCISEARCH

TOTAL FOR ALL FILES
L177         115 L166 AND (BIND? OR BOUND)

=> dup rem l177
PROCESSING COMPLETED FOR L177
```

L178 49 DUP REM L177 (66 DUPLICATES REMOVED)

=> d 1-49 bib abs; fil ca,caplus; d que l57

L178 ANSWER 1 OF 49 MEDLINE DUPLICATE 1
AN 94275393 MEDLINE
TI Eosinophil adhesion to nasal polyp endothelium is P-selectin-dependent.
AU Symon F A; Walsh G M; Watson S R; Wardlaw A J
CS Department of Respiratory Medicine, University of Leicester Medical School, Glenfield General Hospital, United Kingdom..
SO JOURNAL OF EXPERIMENTAL MEDICINE, (1994 Jul 1) 180 (1) 371-6. Journal code: I2V. ISSN: 0022-1007.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals; Cancer Journals
EM 9409
AB Tissue eosinophilia is a characteristic feature of a number of inflammatory diseases including asthma and nasal polyposis. Eosinophil migration into tissues is controlled in part by interactions between eosinophil adhesion receptors and counter-structures on the vascular endothelium. To determine the receptors used by eosinophils to adhere to vascular endothelium in allergic inflammation we have adapted the Stamper-Woodruff frozen section assay (FSA) to study eosinophil adhesion to nasal polyp endothelium. Immunohistology indicated that intercellular adhesion molecule 1 (ICAM-1), E-selectin and P-selectin were well expressed by nasal polyp endothelium, whereas expression of vascular cell adhesion molecule 1 (VCAM-1) was weak or absent. Unstimulated human peripheral blood eosinophils adhered specifically to nasal polyp endothelium. Adherence was temperature and **divalent** cation-dependent and saturable at cell densities > 5 x 10(6) cells/ml. Eosinophil adhesion was almost completely inhibited by a monoclonal antibody (mAb) against P-selectin and by a chimeric molecule consisting of the **Fc** portion of human IgG and the lectin **binding** domain of P-selectin, which **binds** to the P-selectin ligand on leucocytes. Anti-Mac-1 mAb partially inhibited eosinophil adhesion whereas mAb against E-selectin, L-selectin, ICAM-1, VCAM-1, very late activation **antigen** 4, and lymphocyte function-associated **antigen** 1 had no effect. P-selectin is stored in intracellular granules within the endothelial cell and in vitro is only transiently expressed. To determine if P-selectin was expressed on the membrane of the nasal polyp endothelium we compared P-selectin expression in normal skin and nasal polyps after acetone fixation, which permeabilizes cells, and paraformaldehyde, which only allows staining of membrane expressed receptors. In the skin, good expression was seen with acetone fixation but no expression was seen after paraformaldehyde treatment, whereas in nasal polyps, similar expression was observed with both fixatives. In addition immunofluorescence with confocal microscopy demonstrated lumenal staining of nasal polyp endothelium indicating that P-selectin was located on the surface of endothelial

cells while in skin only an intracellular granular distribution was apparent. Lastly, whereas eosinophils **bound** consistently to nasal polyp endothelium, no **binding** was observed to blood vessels in normal skin further supporting the idea that eosinophils were **binding** to membrane expressed and not intracellular P-selectin. The importance of P-selectin in eosinophil adhesion to nasal polyp endothelium suggests that P-selectin antagonists may be effective at inhibiting eosinophil accumulation at sites of allergic inflammation.

L178 ANSWER 2 OF 49 BIOSIS COPYRIGHT 1996 BIOSIS DUPLICATE 2
AN 93:320324 BIOSIS
DN BA96:28674
TI POSTADSORPTION NEUTRALIZATION OF POLIOVIRUS.
AU VRIJSEN R; MOSSER A; BOEYE A
CS DEP. MICROBIOL. HYGIENE, VRIJE UNIV. BRUSSEL, LAARBEEKLAAN 103, 1090 BRUSSELS, BELG.
SO J VIROL 67 (6). 1993. 3126-3133. CODEN: JOVIAM ISSN: 0022-538X
LA English
AB Nineteen neutralizing murine monoclonal antibodies against poliovirus type 1, including representatives reacting with each of the **antigenic** sites on the virion, were tested for their abilities to neutralize the virus either before or after attachment to susceptible cells. All antibodies neutralized unattached virus; six had reasonable titers of postadsorption neutralization (PAN). Experiments with antibodies lacking PAN activity showed that **Fc**-specific rabbit anti-mouse antibodies could confer PAN activity. PAN was shown to involve the prevention of the cell-mediated conversion of virus to 135S and 80S particles. Evidence that one of the PAN-positive antibodies probably **bound** **bivalently** to preadsorbed virions, whereas a PAN-negative antibody **bound** monovalently, is presented. Two PAN-positive antibodies were added to an excess of virus in suspension, and only one antibody caused the virus to aggregate.

L178 ANSWER 3 OF 49 BIOSIS COPYRIGHT 1996 BIOSIS DUPLICATE 3
AN 93:455007 BIOSIS
DN BA96:99907
TI INHIBITION OF INTERFERON-GAMMA BY AN INTERFERON-GAMMA RECEPTOR IMMUNOADHESIN.
AU HAAK-FRENDSCHO M; MARSTERS S A; CHAMOW S M; PEERS D H; SIMPSON N J; ASHKENAZI A
CS DEP. CELL GENETICS, GENENTECH INC., 460 POINT SAN BRUNO BLVD., SOUTH SAN FRANCISCO, CA 94080-4990, USA.
SO IMMUNOLOGY 79 (4). 1993. 594-599. CODEN: IMMUAM ISSN: 0019-2805
LA English
AB Interferon-.gamma. (IFN-.gamma.) is an important cytokine which regulates inflammatory and immune response mechanisms. IFN-.gamma. enhances the presentation and recognition of **antigens** by inducing the expression of major histocompatibility complex (MHC) proteins, by activating effector T cells and mononuclear phagocytes, and by modulating immunoglobulin production and class selection in B cells. Inappropriate production of IFN-.gamma. has been implicated in

the pathogenesis of several autoimmune and inflammatory diseases and in graft rejection. Here, we describe a recombinant inhibitor of IFN-.gamma., termed murine IFN-.gamma. receptor immunoadhesin (mIFN-.gamma.R-IgG). We constructed this immunoadhesin by linking the extracellular portion of the mouse IFN-.gamma.R to the hinge and **Fc** region of an IgG1 heavy chain. Murine IFN-.gamma.R-IgG is secreted by transfected cells as a disulphide-bonded homodimer which **binds** IFN-.gamma. **bivalently**, with high affinity and in a species-specific manner. In vitro, mIFN-.gamma.RIgG can block mIFN-.gamma.-induced antiviral activity and expression of the class I MHC **antigen** H-2Kk in cultured cells. In vivo, mIFN-.gamma.R-IgG can block the function of endogenous mIFN-.gamma. in mouse models of infection with Listeria monocytogenes and of contact sensitivity. These results show that mIFN-.gamma.R-IgG is an effective and specific inhibitor of mIFN-.gamma. both in vitro and in vivo. Thus, in general, IFN-.gamma. receptor immunoadhesins may be useful for investigating the biological functions of IFN-.gamma. as well as for preventing deleterious effects of IFN-.gamma. in human disease.

L178 ANSWER 4 OF 49 WPIDS
AN 92-408470 [50] WPIDS
DNC C92-181172
TI Conjugates comprising **binding** molecules specific for different T-lymphocyte epitope(s) - induce lymphokine production of T-cells, used as immunomodulants, for treating cancers and infectious diseases.
DC B04
IN CHANG, T W; CHANG, T
PA (TANO-N) TANOX BIOSYSTEMS INC
CYC 22
PI EP 516953 A1 921209 (9250)* EN 16 pp
R: AT BE CH DE DK ES FR GB GR IT LI LU MC NL PT SE
CA 2065658 A 921020 (9302)
AU 9214973 A 921217 (9306)
ZA 9202825 A 921230 (9307) 35 pp
AU 642593 B 931021 (9349)
JP 06072895 A 940315 (9415) 12 pp
AU 9453815 A 940324 (9417)
AU 658783 B 950427 (9525)
ADT EP 516953 A1 EP 92-106666 920416; CA 2065658 A CA 92-2065658 920409; AU 9214973 A AU 92-14973 920416; ZA 9202825 A ZA 92-2825 920416; AU 642593 B AU 92-14973 920416; JP 06072895 A JP 92-126764 920420; AU 9453815 A Div ex AU 92-14973 920416, AU 94-53815 940117; AU 658783 B Div ex AU 92-14973 920416, AU 94-53815 940117
FDT AU 642593 B Previous Publ. AU 9214973; AU 658783 B Previous Publ. AU 9453815
PRAI US 91-688000 910419; US 92-819449 920110
AN 92-408470 [50] WPIDS
AB EP 516953 A UPAB: 931116
The following are claimed: (A) mixt. of two or more classes of uncongugated **divalent binding** mols. that have no **Fc** portion, where each class is specific for a different

monovalent T-cell **antigenic** epitope; (B) a bispecific F(ab')2 fragment with specificities for two different monovalent epitopes on the same T-cell **antigen**;(C) conjugate comprising a polymer backbone or microbead coupled to several **binding** mols. specific for a T-cell **antigen**; (D) a mixt. of two or more conjugates, each comprising a polymer backbone coupled to one class of **binding** mol. where each class is specific for a different monovalent epitope on the same T-cell **antigen**; (E) a conjugate comprising a polymer backbone coupled to several different **binding** mols. each **binding** non-competitively to monovalent epitopes on the same T-cell **antigen**; (F) a liposome conjugated on its surface with two or more **binding** mols. each **binding** noncompetitively to different monovalent epitopes on the same T-cell **antigen**; (G) a **divalent binding** mols. which has no **Fc** portion and which **binds** to the CD3-delta **binding** mol. to the same dimer; (H) a conjugate comprising a liposome coupled to several **binding** mols. some of which are specific for one monovalent epitope on a T-cell **antigen** and some of which are specific for another such epitope on the same **antigen**; (I) a conjugate comprising a liposome coupled to several **binding** mols. specific for a T-cell **antigen**.

USE - Used as immunoregulatory substances which induce T-cell activation, proliferation an d/or lymphokine prodn. They may be used as immunostimulants in the treatment of cancer and (esp. viral) infections, or as vaccine adjuvant

Dwg.0/10

L178 ANSWER 5 OF 49 BIOSIS COPYRIGHT 1996 BIOSIS DUPLICATE 4
AN 92:412223 BIOSIS
DN BA94:75423
TI AGGREGATION OF IGE RECEPTOR COMPLEXES ON RAT BASOPHILIC LEUKEMIA CELLS DOES NOT CHANGE THE INTRINSIC AFFINITY BUT CAN ALTER THE KINETICS OF THE LIGAND-IGE INTERACTION.
AU POSNER R G; LEE B; CONRAD D H; HOLOWKA D; BAIRD B; GOLDSTEIN B
CS LOS ALAMOS NATIONAL LAB., LOS ALAMOS, NEW MEXICO 87545.
SO BIOCHEMISTRY 31 (23). 1992. 5350-5356. CODEN: BICHAW ISSN: 0006-2960
LA English
AB The aggregation of IgE anchored to high-affinity **Fc** .epsilon. receptors on rat basophilic leukemia (RBL) cells by multivalent **antigens** initiates transmembrane signaling and ultimately cellular degranulation. Previous studies have shown that the rate of dissociation of **bivalent** and multivalent DNP ligands from RBL cells sensitized with anti-DNP IgE decreases with increasing ligand incubation times. One mechanism proposed for this effect is that when IgE molecules are aggregated, a conformational change occurs that results in an increase in the intrinsic affinity of IgE for **antigen**. This possibility was tested by measuring the equilibrium constant for the **binding** of monovalent DNP-lysine to anti-DNP IgE under two conditions, where the cell-**bound** IgE is dispersed and where it has been aggregated into visible patches on the cell surface using anti-IgE

and a secondary antibody. No difference in the equilibrium constant in these two cases was observed. We also measured the rate of dissociation of a monovalent ligand from cell surface IgE under these two conditions. Whereas the affinity for monovalent ligand is not altered by IgE aggregation, we observe that the rate of ligand dissociation from IgE in clusters is slower than the rate of ligand dissociation from unaggregated IgE. These results are discussed in terms of recent theoretical developments concerning effects of receptor density on ligand **binding** to cell surfaces.

L178 ANSWER 6 OF 49 BIOSIS COPYRIGHT 1996 BIOSIS DUPLICATE 5
AN 92:305926 BIOSIS
DN BA94:19076
TI **BINDING** PARAMETERS AND IDIOTYPIC PROFILE OF THE WHOLE IMMUNOGLOBULIN AND FAB' FRAGMENTS OF MURINE MONOCLONAL ANTIBODY TO DISTINCT DETERMINANTS OF THE HUMAN HIGH MOLECULAR WEIGHT-MELANOMA ASSOCIATED ANTIGEN.
AU TEMPONI M; GOLD A M; FERRONE S
CS DEP. MICROBIOL. AND IMMUNOL., N.Y. MED. COLL., VALHALLA, N.Y. 10595.
SO CANCER RES 52 (9). 1992. 2497-2503. CODEN: CNREA8 ISSN: 0008-5472
LA English
AB The nonspecific accumulation of radioactivity in bone marrow, liver, and spleen in patients with melanoma injected with radiolabeled whole IgG of anti-high molecular weight-melanoma associated **antigen** (HMW-MAA) monoclonal antibody (mAb) hampers the application of immunoscintigraphy to visualize melanoma lesions. This nonspecific background can be reduced by utilizing fragments which do not contain the **Fc** portion of anti-HMW-MAA mAb. Since the in vivo targeting to melanoma lesions of radiolabeled F(ab')2 and Fab' fragments of anti-HMW-MAA mAb is critically dependent on their **binding** parameters, we have analyzed the effect of fragmentation on the **binding** characteristics of the anti-HMW-MAA mAbs 225.28, 763.74, and TP41.2. The three mAbs recognize distinct and spatially distant determinants with a heterogeneous distribution on the pool of HMW-MAA molecules synthesized by melanoma cells. The determinant recognized by mAb TP41.2 is detectable on a markedly smaller population of HMW-MAA molecules than those recognized by mAbs 225.28 and 763.74. 125I-labeled F(ab')2 fragments of the three mAbs displayed an immunoreactive fraction similar to that of the whole IgG, while Fab' fragments displayed a lower one. Fragmentation of mAbs 225.28 and TP41.2 to F(ab')2 produced a 2- and 1.5-fold reduction in their association constants but did not cause a significant change in that of mAb 763.74. Cleavage of F(ab')2 fragments to Fab' fragments produced 2-, 40-, and 7-fold reductions in the association constants of mAbs 225.28, 763.74, and TP41.2, respectively. These changes qualitatively fit the predictions of theory for univalent and **bivalent** mAb **binding**, since mAbs 763.74 and TP41.2 appear to show **bivalent binding** to melanoma cells and mAb 225.28 to show univalent **binding**. The affinity constant of IgG, F(ab')2 and Fab' fragments of mAbs 225.28, 763.74, and TP41.2 displays an inverse relationship with the extent of their time-dependent release from the membrane of melanoma cells. Since no

endocytosis of mAb could be detected, the latter results suggest that radioactivity remains **bound** to melanoma cells in vivo for a longer time following injection of F(ab')2 fragments than following that of Fab' fragments of each of the anti-HMW-MAA mAb tested. Radiolabeled Fab' fragments of mAbs 763.74 and TP41.2 displayed a marked reduction in their reactivity with some of the antiidiotypic mAb tested. The loss of some idiotypes is likely to be caused by changes in the conformation of the molecules associated with the fragmentation of IgG and by damage during the iodination procedure.

L178 ANSWER 7 OF 49 WPIDS COPYRIGHT 1996 DERWENT INFORMATION LTD
AN 91-327158 [45] WPIDS
CR 86-101751 [16]
DNC C91-141267
TI New biologically useful conjugates - contain a paramagnetic or radionuclide metal ion and a biologically useful molecule and are used in in-vivo diagnostics, etc.zoate in solvent.
DC B03
PA (MLCW) MALLINCKRODT INC
CYC 10
PI EP 455268 A 911106 (9145)*
R: AT BE CH DE FR GB IT LI NL SE
ADT EP 455268 A EP 91-110020 910619
PRAI US 84-659456 841010
AN 91-327158 [45] WPIDS
CR 86-101751 [16]
AB EP 455268 A UPAB: 940803
A biologically useful conjugate (1) is claimed which is obtd. by joining a paramagnetic or radionuclide metal ion with a biologically useful molecule (other than a Fab' antibody fragment) using a coupling agent of formula (i); R = a divalent organic radical, e.g. -(CH2)n-(n = 1-20), esp. -C7H14- or -C5H10, or phenylene; and R1 = a gp. able to chelate a paramagnetic radionuclide metal ion, e.g., -N(CH2CH2N(CH2CO2H)2)2 and -CH(N(CH2CO2H)2)CH2N(CH2CO2H)2

Also claimed is a method of making (1) as above.

USE/ADVANTAGE - (1) is used in in vivo diagnostics as an imaging agent and to treat malignant tumours. Suitable antibodies sued include known effective tumour markers, e.g. carcinoembryonic, alpha-foetoprotein, human chorionic gonadoptropin or its beta subunit, colon-specific antigen-p and tumour-specific glycoprotein. @(16pp
ABEQ DE 3586479 G UPAB: 930928
Coupling agent of formula (I) is useful for joining a paramagnetic or radionuclide metal ion with a biologically active material (II). R=**divalent** organic radical; R'=gp capable of chelating a paramagnetic or radionuclide metal ion.

USE/ADVANTAGE - (I) are used to give coupled prods. for diagnostic and erapeutic uses, esp. for treating malignant tumours or for in vivo diagnostic imaging. When (II) is an antibody, (I) is reacted with the free SH gp of the Fab' fragment, so that the **antig n-binding** capacity is retained. (I) may also react with the amino gps in (II) under approp. conditions, but **antigen-binding** capacity may be reduced. The

conjugates for therapeutic or diagnostic use are administered intravenously in buffer soln.

ABEQ EP 178125 B UPAB: 930928

An antibody-paramagnetic ion conjugate of the formula (III), wherein Ab is the residue of a Fab' fragment of an antibody which retains **antigen-binding** activity following enzymatic removal of the **Fc** fragment and reduction cleavage of the disulphide bond joining the heavy chains; R is a **divalent** organic linker; and R'' represents a chelate complex between a paramagnetic metal ion and a group R' which is a group capable of chelating a paramagnetic metal ion.

0/1

L178 ANSWER 8 OF 49 EMBASE COPYRIGHT 1996 ELSEVIER SCI. B.V.

AN 91327242 EMBASE

TI A humanized monovalent CD3 antibody which can activate homologous complement.

AU Routledge E.G.; Lloyd I.; Gorman S.D.; Clark M.; Waldmann H.

CS Division of Immunology, Department of Pathology, Cambridge University, Tennis Court Road, Cambridge CB2 1QP, United Kingdom

SO EUR. J. IMMUNOL., (1991) 21/11 (2717-2725).
ISSN: 0014-2980 CODEN: EJIMAF

CY Germany, Federal Republic of

DT Journal

FS 016 Cancer
022 Human Genetics
025 Hematology
026 Immunology, Serology and Transplantation
029 Clinical Biochemistry

LA English

SL English

AB The rat monoclonal antibody (mAb) YTH12.5, specific for the CD3 **antigen** complex on human T cells has been modified in order to improve its efficacy in human therapy. With the aim of rendering it less immunogenic, it has been humanized using the method of framework grafting. During this process sequence analysis of the YTH12.5 V(L) gene indicated that it was of the .lambda. subclass, however, it was markedly dissimilar from previously published rat and mouse V(.lambda.) gene sequences and may represent a new V.lambda. gene family. The humanization of this light chain represents the first successful reshaping of a .lambda. light chain V region. To improve the effector function of the antibody we have created a monovalent form (1 Fab, 1 **Fc**) using a novel method involving the introduction of an N-terminally truncated human IgG1 heavy chain gene into cells producing the humanized CD3 mAb. Comparison of the mono- and **bivalent** humanized mAb in a complement-mediated cell lysis assay revealed that the monovalent antibody mediated lysis of human T cell blasts whereas the **bivalent** form did not. The availability of a humanized, complement-fixing CD3 mAb may improve opportunities for human therapy, in the management of organ rejection, autoimmunity and the treatment of T cell lymphoma.

L178 ANSWER 9 OF 49 WPIDS COPYRIGHT 1996 DERWENT INFORMATION LTD
AN 90-343194 [46] WPIDS
CR 86-077233 [12]; 90-016314 [03]
DNN N90-262450 DNC C90-148768
TI Antibody-radionuclide conjugated prepn. - from fab fragment of mono clonal antibody linked through maleimide coupler to chelating moiety.
DC B04 K08 S03
IN KETRING, A R; NICOLOTTI, R A; PAKKOON, Y
PA (MLCW) MALLINCKRODT INC
CYC 10
PI EP 397213 A 901114 (9046)*
R: AT BE CH DE FR GB IT LI NL SE
ADT EP 397213 A EP 85-111068 850912
PRAI US 84-650127 840913
AN 90-343194 [46] WPIDS
CR 86-077233 [12]; 90-016314 [03]
AB EP 397213 A UPAB: 940803
Method for prepg. an antibody-radionuclide conjugate (I) derived from an antibody conjugate of formula (II) where Ab = residue of a Fab' fragment of a monoclonal antibody which retains **antigen -binding** activity following enzymatic removal of the **Fc** fragment and reductive clearage of the disulphide bond joining the heavy chains; R= **divalent** organic linker; and R'= gp. capable of chelating a radionuclide metal ion and having agreater chelating affinity than 4,5-dihydroxy-m-benzene disulphonic acid (III) for the radionuclide ion; and from a radionuclide metal ion, comprises reacting a radionuclide metal ion transfer couplex comprising a chelate of (III) or salt and a radionuclide metal ion with the antibody conjugate, reaction being in aq. medium at a pH at h)which the antibody conjugate is stable.
USE ADVANTAGE - Useful in therapy e.g. in treatment of malignant tumours, or as a diagnostic imaging agent. The antibody fragments may be better able to penetrate to the target site, and may minimise problems of immunogenicity and cross-reactivity associated with whole antibodies. The labelling site is distant from the **antigen-binding** site, so conjugated coupling agent does not interfere with **antigen binding**.
@(17pp Dwg.No.1/1)@
1/1@

L178 ANSWER 10 OF 49 BIOSIS COPYRIGHT 1996 BIOSIS DUPLICATE 6
AN 90:350913 BIOSIS
DN BA90:47492
TI AN ALTERNATIVE LEUKOCYTE HOMOTYPIC ADHESION MECHANISM LFA-1-ICAM-1-INDEPENDENT TRIGGERED THROUGH THE HUMAN VLA-4 INTEGRIN.
AU CAMPANERO M R; PULIDO R; URSA M A; RODRIGUEZ-MOYA M; DE LANDAZURI M O; SANCHEZ-MADRID F
CS SERVICIO DE IMMUNOL., HOSPITAL DE LA PRINCESA, UNIV. AUTONOMA DE MADRID, DIEGO DE LEON, 62.28006 MADRID, SPAIN.
SO J CELL BIOL 110 (6). 1990. 2157-2166. CODEN: JCLBA3 ISSN: 0021-9525
LA English
AB The VLA-4 (CD49d/CD29) integrin is the only member of the VLA family

expressed by resting lymphoid cells that has been involved in cell-cell adhesive interactions. We here describe the triggering of homotypic cell aggreation of peripheral blood T lymphocytes and myelomonocytic cells by mAbs specific for certain epitopes of the human VLA.alpha.4 subunit. This anti-VLA-4-induced cell adhesion is isotype and **Fc** independent. Similar to phorbol ester-induced homotypic adhesion, cell aggregation triggered through VLA-4 requires the presence of **divalent** cations, integrity of cytoskeleton and active metabolism. However, both adhesion phenomena differed at their kinetics and temperature requirements. Moreover, cell adhesion triggered through VLA-4 cannot be inhibited by cell preincubation with anti-LFA-1.alpha. (CD11a), LFA-1.beta. (CD18), or ICAM-1 (CD54) mAb as opposed to that mediated by phorbol esters, indicating that it is a LFA-1/ICAM-1 independent process. Antibodies specific for CD2 or LFA-3 (CD58) did not affect the VLA-4-mediated cell adhesion. The ability to inhibit this aggregation by other anti-VLA-4-specific antibodies recognizing epitopes on either the VLA.alpha.4 (CD49d) or .beta. (CD29) chains suggests that VLA-4 is directly involved in the adhesion process. Furthermore, the simultaneous **binding** of a pair of aggregation-inducing mAbs specific for distinct **antigenic** sites on the .alpha.4 chain resulted in the abrogation of cell aggregation. These results indicate that VLA-4-mediated aggregation may constitute a novel leukocyte adhesion pathway.

L178 ANSWER 11 OF 49 BIOSIS COPYRIGHT 1996 BIOSIS DUPLICATE 7
AN 91:56345 BIOSIS
DN BA91:34626
TI MURINE IMMUNOGLOBULIN G ANTI-CD4 MONOCLONAL ANTIBODIES **BIND** TO PRIMARY HUMAN MONOCYTES AND MACROPHAGES THROUGH FC RECEPTORS AS WELL AS AUTHENTIC CD4.
AU TEPPLER H; LEE S-H; RIEBER E P; GORDON S
CS SIR WILLIAM DUNN SCH. PATHOL., UNIV. OXFORD, SOUTH PARKS RD., OXFORD OX1 3RE, UK.
SO AIDS (PHILA) 4 (7). 1990. 627-632. CODEN: AIDSET ISSN: 0269-9370
LA English
AB Using Western blot and fluorescence-activated cell sorter (FACS) analysis, we demonstrated that primary human monocytes and cultured-derived macrophages express low levels of authentic CD4 **antigen**. The plasma membrane **Fc** receptor (**FcR**) on mononuclear phagocytes plays a major role in the **binding** of murine immunoglobulin G2.alpha. anti-CD4 monoclonal antibody (MAb), and contributes to **binding** of other subclasses. The **FcR** detected shows an increase in apparent molecular weight from 60 to 70 kD over 2 weeks in culture. U937 cells resemble T lymphocytes, rather than primary monocytes/macrophages, in expressing relatively high levels of CD4; **FcR** contributes little to the signal. The potential **bivalent** interactions between immunoglobulin G and receptors such as CD4 and **FcR** could influence the **binding** and fate of HIV in primary monocytes/macrophages.

L178 ANSWER 12 OF 49 MEDLINE DUPLICATE 8

AN 90383260 MEDLINE
TI Site-directed immobilization of proteins.
AU Domen P L; Nevens J R; Mallia A K; Hermanson G T; Klenk D C
CS Pierce Chemical Co., Rockford, IL 61105..
SO JOURNAL OF CHROMATOGRAPHY, (1990 Jun 27) 510 293-302.
Journal code: HQF. ISSN: 0021-9673.
CY Netherlands
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 9012
AB To determine if immobilization chemistry can be used to orient antibody on a support so that the **bivalent binding** potential can be fully utilized, we developed three activated matrices that couple to different functional groups on the molecule. When AminoLink Gel was used to couple antibody randomly through primary amino groups, the molar ratio of immobilized antibody to recovered **antigen** averaged 1:1. Iodoacetyl groups on SulfoLink Gel couple through sulfhydryls in the hinge region of the antibody molecule, in theory leaving the **antigen binding** site available. However, the antibody-to-**antigen** molar ratio was only slightly improved. Hydrazide groups on CarboLink Gel couple to aldehyde groups generated by oxidation of carbohydrate moieties that are located primarily on the **Fc** portion of the antibody molecule. The molar ratio of immobilized antibody to purified **antigen** using CarboLink Gel reached the optimum of 1:2. CarboLink Gel is most effective at orienting antibody for better **antigen** purification capability.

L178 ANSWER 13 OF 49 MEDLINE
AN 91033895 MEDLINE
TI CD45 molecule cross-linking inhibits natural killer cell-mediated lysis independently of lytic triggering.
AU Starling G C; Hart D N
CS Department of Haematology, Christchurch Hospital, New Zealand..
SO IMMUNOLOGY, (1990 Oct) 71 (2) 190-5.
Journal code: GH7. ISSN: 0019-2805.
CY ENGLAND: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals; Cancer Journals
EM 9102
AB The fact that certain CD45 [anti-leucocyte common **antigen** (LCA)] monoclonal antibodies (mAb) inhibit natural killer (NK) cell non-major histocompatibility complex (MHC)-restricted cytolysis led to the suggestion that these mAb block a 'trigger' for NK cell lytic activity. However, the discovery that the intracytoplasmic portion of the leucocyte common molecule has protein tyrosine phosphatase activity raises the possibility that the mAb initiate a direct inhibitory signal, independent of the triggering apparatus. To clarify this, we have tested the ability of CD45 antibodies to trigger NK cells and redirect cytotoxicity against mAb-producing

hybridoma cells and autologous monocytes, an approach which has identified other cytotoxic trigger molecules. Peripheral blood NK cells failed to kill the CD45 antibody-producing hybridomas, although a CD3 antibody expressing hybridoma was susceptible to cytotoxic T-cell lysis. Furthermore, the CD45 mAb CMRF-12 + 26, 13.3 and HuLyM4 did not redirect lysis of autologous monocytes by NK cells, whereas the isotype-matched **CD16** mAb did so.

Bivalent CD45 antibody was necessary to block NK lysis of K562, as F(ab')2 but not F(ab') fragments of CMRF-12 + 26 antibody inhibited killing. Capping of the LCA appeared to correlate with the ability of the CD45 mAb to block killing, suggesting that cross-linking of LCA molecular isoforms on the NK cell surface is required for CD45 mAb to inhibit non-MHC-restricted cytolysis.

L178 ANSWER 14 OF 49 DISSABS COPYRIGHT 1996 UMI
AN 89:4529 DISSABS Order Number: AAR0564536 (not available for sale by UMI)
TI A STUDY OF THE CELLULAR INTERACTIONS AND GENETIC RESTRICTIONS IN THE INDUCTION OF ANTISUPPRESSION
AU UNIYAL, SHASHI [PH.D.]
CS THE UNIVERSITY OF MANITOBA (CANADA) (0303)
SO Dissertation Abstracts International, (1989) Vol. 49, No. 11B, p. 4747. Order No.: AAR0564536 (not available for sale by UMI).
DT Dissertation
FS DAI
LA English
AB Antisuppression is an immunoregulatory T cell pathway activated within 6 hours after **antigenic** stimulation which enhances immune responses through the inhibition of suppressor cell function. The antisuppressor mediator, present in the serum of mice at 6 hours (6HS), contains a unique form of processed **antigen** which represents complexes of immunoglobulin (Ig) and **antigen**. The formation of these complexes is mediated by an inducer Ly1$\sp+$ L3T4$\sp+$T cell while their function depends on **binding** to and activating an Ly2$\sp+$ effector T cell. Results from this study have shown that the initial interaction between macrophages and T cells for the in vitro production of this mediator requires **antigen** processing and presentation in association with major histocompatibility complex (MHC) class II molecules to an Ly1$\sp+$, L3T4$\sp+$ inducer T cell. Inhibition of **antigen** uptake and its subsequent degradation abolished the production of this mediator. Its generation was also blocked in the absence of cell contact between the macrophage and T cell by both anti-L3T4 and anti-class II antibodies.

Lethally irradiated mice required reconstitution with syngeneic T cells in order to generate the antisuppressor mediator. When Igh congenic T cells were used for reconstitution the antisuppressor mediator was lacking. However, the addition of IgG from the same Igh haplotype as the T cell donor generated antisuppressor complexes that enhanced IgG antibody response in suppressed cultures. The Igh restriction between the inducer T cell and IgG was observed in several Igh congenic strains. The inducer T cell was unable to express an altered Igh restriction when exposed to an Igh distinct

environment for several days. By using two Igh recombinant strains it was found that the formation of this mediator was regulated by genes of the IghV locus. In addition, it was observed that genes of the IghC locus were not involved in the formation and functional expression of antisuppression. In the absence of the **Fc** portion of IgG, the **divalent** F(ab$\sp\prime$)$\sb2$ fragment participated in the formation of functional complexes. The effector stage of antisuppression was found to be MHC and Igh nonrestricted.

L178 ANSWER 15 OF 49 DISSABS COPYRIGHT 1996 UMI
AN 89:23001 DISSABS Order Number: AAR9005958
TI EVENTS IN B CELLS DURING ANTIGEN PRESENTATION
AU WEBER, DOMINIQUE ANTONELLI [PH.D.]
CS COLUMBIA UNIVERSITY (0054)
SO Dissertation Abstracts International, (1989) Vol. 50, No. 10B, p. 4445. Order No.: AAR9005958. 137 pages.
DT Dissertation
FS DAI
LA English
AB The purpose of this research is to elucidate the events occurring in B cells between the time of encounter with **antigen** and the display of an immunogenic complex (**antigen** peptide- Class II MHC molecule) on the B cell surface. This **antigen** presentation is the first step in the development of an immune response.

Antiimmunoglobulin antibodies can function as T-dependent **antigens**, they **bind** to the **antigen** receptor (mIg) of B cells, are internalized, processed and presented to a primed T cell population. B cells present monovalent or **divalent** antibodies to mIg and it has been shown that the **divalent** ligands are presented with a higher efficiency, which can be correlated to the degree of activation of the cells. The involvement of the surface **Fc** receptor of the B cell, together with the **binding** of a ligand to the immunoglobulin receptor, has a negative effect on the **antigen** presentation ability of the cell. The cross-linking of **antigen-Fc**-receptors is similar to the interactions occurring with **antigen**-antibody complexes on the B cell surface and points to a role of the **Fc** receptor in the regulation of the immune response at the level of the B cell. B cells can present both ligands to their immunoglobulin isotypes IgD and IgM.

Activated B lymphocytes internalize and recycle their membrane MHC Class II receptors. This is a specific phenomenon since they do not internalize their membrane Class I. Measurements of immunofluorescence and fluorescence quenching show that Class II molecules are rapidly internalized into an acidic compartment. This internalization is not induced by the **binding** of the antibody to Class II, and therefore seems to occur spontaneously in activated B cells.

B cells internalize both monovalent and **divalent** antibodies to their mIg receptors, into acidic endosomes which fuse

extensively with vesicles containing internal Class II molecules, yet a small proportion of these vesicles does not associate with **antigen**. Crosslinking of mIg receptors induces an increased uptake of **antigen** but does not induce internalization of membrane Class II, which occurs independently of **antigen binding**. The vesicles containing both **antigen** and Class II molecules may be the site of **antigen** peptide-MHC complex formation.

L178 ANSWER 16 OF 49 BIOSIS COPYRIGHT 1996 BIOSIS DUPLICATE 9
AN 89:427215 BIOSIS
DN BA88:85473
TI TRYPTIC DIGESTION OF XENOPUS IGM AND IGY MOLECULES.
AU COOSEMANS V; HADJI-AZIMI I; PERRENOT N
CS STATION DE ZOOLOGIE EXPERIMENTALE, 154 ROUTE DE MALAGNOU, CH-1224 CHENE-BOUGERIES/SWITZERLAND.
SO DEV COMP IMMUNOL 13 (2). 1989. 149-158. CODEN: DCIMDQ ISSN: 0145-305X
LA English
AB Xenopus IgM and IgY molecules were digested by trypsin. Their respective fragments were separated by gel filtration and immunoadsorption. The purified fragments were characterized by SDS-PAGE and immunoblotting. Tryptic digestion of Xenopus IgM resulted in the release, at a low yield, of hexameric **Fc** .mu., and of monovalent Fab.mu. fragments. The digestion of Xenopus IgY antibodies led to the recovery of **divalent** and monovalent Fab .nu. fragments. The **antigen-binding** property of these fragments was demonstrated. No **Fc** .nu. fragments of appreciable size could be detected.

L178 ANSWER 17 OF 49 MEDLINE DUPLICATE 10
AN 90158552 MEDLINE
TI Attack on neoplastic cell membranes by therapeutic antibody.
AU Stevenson G T
CS Lymphoma Research Unit, Tenovus Laboratory, General Hospital, Southampton, UK..
SO MOLECULAR AND CELLULAR BIOCHEMISTRY, (1989 Nov 23-Dec 19) 91 (1-2) 33-8.
Journal code: NGU. ISSN: 0300-8177.
CY Netherlands
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 9005
AB Mouse monoclonal antibody is not well fitted to destroying tumour cell targets. Complement and cellular effectors are inefficiently recruited, the cells can undergo **antigenic** modulation, **antigen**-negative mutants can arise, and the tumour-bearing subject can amount an immune response against the therapeutic antibody. This paper describes the preparation of two chimeric antibody derivatives designed to cirvumvent some of these problems. The first derivative is FabFc, prepared by linking Fab' gamma from monoclonal antibody to **Fc** gamma from human IgG. The

bismaleimide linking agent forms a thioether bond with an SH group released by reduction of SS bonds in the hinge of each constituent. The second derivative is bisFabFc, formed by a bismaleimide in this case joining two FabFc molecules via a free SH in the **Fc** hinge of each. As regards antibody activity against target cells bisFabFc can be univalent (one active, one inactive Fab arm), **bivalent**, or bispecific (with each Fab arm directed against a different cell surface **antigen**). Its juxtaposed dual **Fc** regions are designed to promote cooperative **binding** of effectors. Some preliminary characterization in vitro has employed antibodies of anti-idiotypic specificity directed against guinea-pig L2C leukaemic B lymphocytes. The parent mouse IgG1 antibody failed to invoke complement cytotoxicity or antibody-dependent cellular cytotoxicity, while the chimeric derivatives yielded good killing in both systems. In complement lysis **bivalent** bicFabFc outperformed univalent, which in turn outperformed the FabFc monomer.

L178 ANSWER 18 OF 49 LIFESCI COPYRIGHT 1996 CSA
AN 89:9368 LIFESCI
TI Radiolabeled antibody fragments.
AU Nicolotti, R.A.
CS Mallinckrodt, Inc., St. Louis, MO (USA)
PI US 4837003 1989
SO (1989) . US Cl. 424/1.1; Int. Cl. G01N 33/534, 33/563..
DT Patent
FS W
LA English
AB The formula of an antibody conjugate is given, wherein Ab is the residue of a Fab' fragment of a monoclonal antibody, said antibody being one which retains **antigen-binding** activity following enzymatic removal of the **Fc** fragment and reductive cleavage of the disulfide bond joining the heavy chains. It also contains: the residue of the free sulfhydryl group of the Fab' fragment; a **divalent** organic linker; and a nontoxic, weakly acidic chelating group capable of forming a chelate with a radionuclide metal ion that is thermodynamically stable under physiological conditions which has a higher stability constant than a chelate of the radionuclide with transferrin in vivo.

L178 ANSWER 19 OF 49 BIOSIS COPYRIGHT 1996 BIOSIS DUPLICATE 11
AN 89:94540 BIOSIS
DN BA87:48676
TI A MONOCLONAL ANTIBODY THAT INHIBITS SECRETION FROM RAT BASOPHILIC LEUKEMIA CELLS AND **BINDS** TO A NOVEL MEMBRANE COMPONENT.
AU SOTO E O; PECHT I
CS DEP. CHEM. IMMUNOL., WEIZMANN INST. SCI., REHOVOT 76100, ISRAEL.
SO J IMMUNOL 141 (12). 1988. 4324-4332. CODEN: JOIMA3 ISSN: 0022-1767
LA English
AB Rat basophilic leukemia [RBL-2H3] cells, like mast cells and basophils, carry monovalent membrane receptors with high affinity for IgE [**FcR**]. Cross-linking of these receptors provides the immunologic stimulus which initiates a series of biochemical events,

culminating in secretion of inflammatory mediators. In an attempt to identify membrane component involved in the stimulus-secretion coupling of these cells, hybridomas were produced from splenocytes of mice immunized with intact RBL-2H3 cells. Here we report the production of a mAb [designated G63] that inhibits the **FcR** -mediated secretion from RBL cells. At low degrees of **FcR** aggregation, the mAb G63-induced inhibition may be complete, whereas at the maximum of secretion the inhibition is in the range of 30 to 40%. The relative degree of inhibition of secretion is dependent on the dose of mAb G63. Furthermore, inhibition requires the **bivalency** of G63, as the Fab fragments are inactive. The number of **antigenic** epitopes recognized by G63 per RBL-2H3 cell is 1.8 .times. 104 epitopes/cell, as determined by direct **binding** studies of 125I-labeled Fab fragments of G63. This number is 20 to 30 times smaller than that of **FcR** on the same cells. The membrane component to which G63 **binds** has been identified by immunoprecipitation as a glycoprotein with an apparent Mr of 58 to 70 kDa. All of these results, and the fact that no competition for **binding** to RBL cells between mAb G63 and IgE can be resolved, indicate that mAb G63 **binds** to a membrane component which is distinct from the **FcR**. mAb G63 suppresses the **FcR**-mediated rise in cytoplasmic concentration of free Ca2+ ions, known to be one of the biochemical signals involved in the stimulus-secretion coupling in RBL-2H3 cells. G63 does not affect, however, the degranulation induced by the Ca2+ ionophore A23187. Therefore, mAb G63 probably exerts its inhibitory effect on a step preceding the rise in cytoplasmic free Ca2+. Thus, mAb G63 defines a previously unidentified membrane component that is involved in one of the early steps of the RBL-2H3 activation mediated by their **FcR**.

L178 ANSWER 20 OF 49 WPIDS COPYRIGHT 1996 DERWENT INFORMATION LTD
AN 87-298888 [43] WPIDS
DNN N87-223582 DNC C87-127188
TI Prepn. of conjugate of protein or polypeptide and metal ion - by using metal ion chelate of 4,5-di hydroxy-m-benzene -di sulphonic acid and chelate of the protein or polypeptide to give reporter mols..
DC B04 K08 S03
IN KETRING, A R; KOON, Y P; NICOLOTTI, R A; PAK, K Y
PA (MLCW) MALLINCKRODT INC
CYC 15
PI AU 8769701 A 870910 (8743)* 37 pp
EP 248506 A 871209 (8749) EN
R: AT BE CH DE ES FR GB IT LI NL SE
JP 62270600 A 871124 (8801)
US 4732974 A 880322 (8815) 12 pp
CA 1288197 C 910827 (9139)
EP 248506 B1 920812 (9233) EN 22 pp
R: AT BE CH DE ES FR GB IT LI NL SE
DE 3781031 G 920917 (9239)
ES 2035041 T3 930416 (9324)
JP 07091317 B2 951004 (9544) 15 pp

ADT AU 8769701 A AU 87-69701 870304; EP 248506 A EP 87-301869 870304; JP 62270600 A JP 87-47871 870304; US 4732974 A US 86-836535 860305; EP 248506 B1 EP 87-301869 870304; DE 3781031 G DE 87-3781031 870304, EP 87-301869 870304; ES 2035041 T3 EP 87-301869 870304; JP 07091317 B2 JP 87-47871 870304

FDT DE 3781031 G Based on EP 248506; ES 2035041 T3 Based on EP 248506; JP 07091317 B2 Based on JP 62270600

PRAI US 86-836535 860305

AN 87-298888 [43] WPIDS

AB AU 8769701 A UPAB: 930922

Prepn. of a conjugate of a protein (I) or polypeptide (II) and a metal ion comprises reacting a metal ion transfer complex comprising a chelate of 4,5-dihydroxy-m-benzene disulphonic acid (III) or its salt and a metal ion with (I) or (II) covalently **bound** to an exogenous chelating gp.. This gp. has a greater chelating affinity than (III) for the metal ion. reaction is effected in aq. medium at a pH value where (I) or (II) is stable.

USE/ADVANTAGE - The conjugates are useful as reporter molecules, as the metal ions can be detected at very low concn. by emission of radioactivity, fluorescence, electron spin resonance and n.m.r. relaxation. They may be attached to carriers such as anti-bodies or antigens for diagnosis of diseases, esp. tumours, and for treatment of the diseases, e.g. of tumours when a radiation-emitting metal ion is used. In an example indium-111 trichloride (10 microlitres; 50-100 microcuries) in 50 mM HCl was added to 10mM (III) (5 microlitres), 4mMHCl and the mixt. was incubated for 5 mins. Then 200mM MES 810 microlitres) adn 2-15 mg/ml conjugate of anti-CEA Fab' and coupling agent (10 microb-litres) were added. The mixt. was incubated for 1 hr. to form the chelate of indium-111 and the conjugate (identified by electrophoresis).

0/1

ABEQ DE 3781031 G UPAB: 930922

Prepn. of a conjugate of a protein (I) or polypeptide (II) and a metal ion comprises reacting a metal ion transfer complex comprising a chelate of 4,5-dihydroxy-m-benzene disulphonic acid (III) or its salt and a metal ion with (I) or (II) covalently **bound** to an exogenous chelating gp.. This gp. has a greater chelating affinity than (III) for the metal ion. reaction is effected in aq. medium at a pH value where (I) or (II) is stable.

USE/ADVANTAGE - The conjugates are useful as reporter molecules, as the metal ions can be detected at very low concn. by emission of radioactivity, fluorescence, electron spin resonance and n.m.r. relaxation. They may be attached to carriers such as anti-bodies or **antigens** for diagnosis of diseases, esp. tumours, and for treatment of the diseases, e.g. of tumours when a radiation-emitting metal ion is used. In an example indium-111 trichloride (10 microlitres; 50-100 microcuries) in 50 mM HCl was added to 10mM (III) (5 microlitres), 4mMHCl and the mixt. was incubated for 5 mins. Then 200mM MES 810 microlitres) adn 2-15 mg/ml conjugate of anti-CEA Fab' and coupling agent (10 microb-litres) were added. The mixt. was incubated for 1 hr. to form the chelate of indium-111 and the conjugate (identified by electrophoresis).

ABEQ EP 248506 B UPAB: 930922

A method of preparing a conjugate of a protein or pplypeptide (other than an antibody conjugate of the formula (I) wherein Ab is the residue of a Fab' fragment of a monoclonal antibody, said antibody being one which retains **antigen-binding** activity following enzymatic removal of the **Fc** fragment and reductive cleavage of the disulphide bond joining the heavy chains; R is a **divalent** linker; and R' is a group which is capable of chelating a radionuclide metal ion) and a metal ion selected from indium, gallium, technetium and isotopes thereof which comprises reacting a metal ion transfer complex comprising a chelate of 4,5-dihydroxyl-m-benzenedisulphonic acid or a salt thereof and said metal ion with said protein or polypeptide, said protein or polypeptide being covalently **bound** to an exogenous chelating group, said exogenous chelating group having a greater chelating affinity than 4,5-dihydroxyl-m-benzenedisulphonic acid for the metal ion, the reaction being carried out in an aqueous medium at a pH at which the protein or polypeptide is stable.
0/2

ABEQ US 4732974 A UPAB: 930922
Prepn. of a conjugate of a protein or polypeptide and a metal ion comprises reacting a metal ion transfer complex comprising a chelate of 4,5-dihydroxy-m-benzenedisulphonic acid or its salt, a metal ion with a protein or polypeptide that is covalently **bound** to an exogenous chelating gp. which has greater chelating affinity than 4,5-dihydroxylm-benzenedisulphonic acid for the metal ion. Reaction takes place in aq. medium at pH at which the protein or polypeptide is stable. Suitably, the protein or polypeptide is an antibody or fragment, esp. to a tumour associated **antigen** or carcinoembryonic **antigen**.

USE - As reporter substances for in vivo and in vitro diagnosis and, for targeted delivery as therapeutic agents if a radionuclide metal ion is used.

L178 ANSWER 21 OF 49 EMBASE COPYRIGHT 1996 ELSEVIER SCI. B.V.
AN 87092927 EMBASE
TI Anti-T3 induced cytotoxicity: The role of target cell Fc-receptors in the lysis of autologous monocytes and the Fc-independent lysis of T3-positive target cells.
AU Leeuwenberg J.F.M.; Lems S.P.M.; Capel P.J.A.
CS Division of Nephrology, University of Nijmegen, St Radboud Hospital, 6525 GA Nijmegen, Netherlands
SO TRANSPLANT. PROC., (1987) 19/1I (428-431).
CODEN: TRPPA8
CY United States
FS 013 Dermatology and Venereology
026 Immunology, Serology and Transplantation
LA English
AB The authors have shown previously that anti-T3 monoclonal antibodies (MAb), in addition to blocking specific CTL target-cell interaction, are able to induce lysis of nonspecific targets. By using F(ab')2 fragments of anti-T3 MAb, induction of lysis was shown to be **Fc**-dependent, and in the analysis of a panel of different target cell types, only those targets with demonstrable **Fc**

-receptors (**FcR**) for murine IgG (sub)classes, were lysed by cytotoxic T lymphocytes (CTL) in the presence of anti-T3 MAb of the appropriate subclass. Anti-CD8, anti-CD4, anti-CD7, and anti-HLA are ineffective in this process, indicating that cell-cell contact via **FcR** bridging of antibody coated CTLs and targets is not sufficient to induce nonspecific lysis. Activation of the lytic machinery of the CTL via T3 crosslinking by **FcR** on the target cells, therefore, is a prerequisite for this process. As an approach to the understanding of the effects of anti-T3 MAb in vivo,we show here that in addition to the lysis of **FcR** -positive cell lines and heterologous monocytes, CTLs are also able to kill autologous monocytes ie, monocytes from the same individual from whom the CTLs were derived. Furthermore, evidence is produced for an **Fc**-independent anti-T3-induced lysis. The latter phenomenon was dependent on the use of T3-positive targets, and this suggests a CTL target-cell bridging via the **binding** of the F(ab')2 parts of the **divalent** IgG molecule to the T3 **antigens** on CTL and target.

L178 ANSWER 22 OF 49 WPIDS COPYRIGHT 1996 DERWENT INFORMATION LTD
AN 86-101751 [16] WPIDS
CR 91-327158 [45]
DNC C86-043530
TI Use of bifunctional maleimide derivs. for coupling antibodies etc. - then for chelating paramagnetic or radionuclide metal ion to give therapeutic or diagnostic prod. esp. for cancer therapy.
DC B03 K08
IN DEAN, R T; NICOLOTTI, R A
PA (MLCW) MALLINCKRODT INC
CYC 15
PI EP 178125 A 860416 (8616)* EN 29 pp
R: AT BE CH DE FR GB IT LI NL SE
AU 8548451 A 860417 (8623)
JP 61093131 A 860512 (8625)
US 4659839 A 870421 (8718)
CA 1257601 A 890718 (8933)
EP 178125 B1 920812 (9233) EN 19 pp
R: AT BE CH DE FR GB IT LI NL SE
DE 3586479 G 920917 (9239)
JP 06047560 B2 940622 (9423) 12 pp
ADT JP 61093131 A JP 85-223860 851009; US 4659839 A US 84-659456 841010; EP 178125 B1 EP 85-307064 851002; DE 3586479 G DE 85-3586479 851002, EP 85-307064 851002; JP 06047560 B2 JP 85-223860 851009
FDT DE 3586479 G Based on EP 178125; JP 06047560 B2 Based on JP 61093131
PRAI US 84-659456 841010
AN 86-101751 [16] WPIDS
CR 91-327158 [45]
AB EP 178125 A UPAB: 940803
Coupling agent of formula (I) is useful for joining a paramagnetic or radionuclide metal ion with a biologically active material (II). R=divalent organic radical; R'=gp capable of chelating a paramagnetic or radionuclide metal ion.
USE/ADVANTAGE - (I) are used to give coupled prods. for

diagnostic and erapeutic uses, esp. for treating malignant tumours or for in vivo diagnostic imaging. When (II) is an antibody, (I) is reacted with the free SH gp of the Fab' fragment, so that the antigen-**binding** capacity is retained. (I) may also react with the amino gps in (II) under approp. conditions, but antigen-**binding** capacity may be reduced. The conjugates for therapeutic or diagnostic use are administered intravenously in buffer soln.

0/0

Dwg.0/0

ABEQ DE 3586479 G UPAB: 930922

Coupling agent of formula (I) is useful for joining a paramagnetic or radionuclide metal ion with a biologically active material (II). R=**divalent** organic radical; R'=gp capable of chelating a paramagnetic or radionuclide metal ion.

USE/ADVANTAGE - (I) are used to give coupled prods. for diagnostic and erapeutic uses, esp. for treating malignant tumours or for in vivo diagnostic imaging. When (II) is an antibody, (I) is reacted with the free SH gp of the Fab' fragment, so that the **antigen-binding** capacity is retained. (I) may also react with the amino gps in (II) under approp. conditions, but **antigen-binding** capacity may be reduced. The conjugates for therapeutic or diagnostic use are administered intravenously in buffer soln.

ABEQ EP 178125 B UPAB: 930922

An antibody-paramagnetic ion conjugate of the formula (III), wherein Ab is the residue of a Fab' fragment of an antibody which retains **antigen-binding** activity following enzymatic removal of the **Fc** fragment and reduction cleavage of the disulphide bond joining the heavy chains; R is a **divalent** organic linker; and R'' represents a chelate complex between a paramagnetic metal ion and a group R' which is a group capable of chelating a paramagnetic metal ion.

0/1

ABEQ US 4659839 A UPAB: 930922

Coupling agent for joining a paramagnetic or radionuclide metal ion with an antibody Fab' fragment comprises a cpd. of formula (I), where R is (CH2)n or phenylene; n is 1-20; and R' is a gp. which can chelate a radionucliole metal ion.

Pref. R is C7H14 or C5H10 and R' is N(CH2CH2N(CH2COOH)2)2 or CH(N(CH2COOH)2) CH2N(CH2COOH)2.

USE/ADVANTAGE - For prodn. of conjugates for therapeutic use, e.g. in tumour treatment, or as vivo diagnostic imaging agents. Antibody immunoreactivity is retained.

L178 ANSWER 23 OF 49 WPIDS COPYRIGHT 1996 DERWENT INFORMATION LTD

AN 86-077233 [12] WPIDS

CR 90-016314 [03]; 90-343194 [46]

DNC C86-032886

TI New antibody conjugates with N-substd.-maleimide derivs. - useful for coupling to radionuclide metal ions without adverse effect on immuno-reactivity in therapy and diagnosis.

DC B04 K08

IN NICOLOTTI, R A
PA (MLCW) MALLINCKRODT INC
CYC 15
PI EP 174853 A 860319 (8612)* EN 31 pp
R: AT BE CH DE FR GB IT LI NL SE
AU 8547376 A 860320 (8619)
JP 61072723 A 860414 (8621)
US 4837003 A 890606 (8928)
EP 174853 B 900711 (9028)
R: AT BE CH DE FR GB IT LI NL SE
DE 3578625 G 900816 (9034)
CA 1276878 C 901127 (9102)
JP 06047557 B2 940622 (9423)
ADT EP 174853 A EP 85-306486 850912; JP 61072723 A JP 85-201927 850913;
US 4837003 A US 84-650127 840913; JP 06047557 B2 JP 85-201927 850913
FDT JP 06047557 B2 Based on JP 61072723
PRAI US 84-650127 840913
AN 86-077233 [12] WPIDS
CR 90-016314 [03]; 90-343194 [46]
AB EP 174853 A UPAB: 940803
Antibody conjugates with N-substd.-maleimide derivs. of formula (I) are new. Ab = residue of a Fab' fragment of monoclonal antibody, the antibody retaining **antigen-binding** activity after enzymatic removal of the **Fc** fragment and reductive cleavage of the disulphide bond joining the heavy chains; R = **divalent** organic linker gp.; R' = gp. capable of chelating a radionuclide metal ion.

Antibody-radionuclide conjugate (II) comprising (I) in which gp. R' has formed a chelate complex with a radionuclide metal ion is new.

USE/ADVANTAGE - (I) are coupled to radionuclide metal ions to give (II) without the immunoreactivity of the antibody being adversely affected. (II) are useful for therapeutic use and for diagnosis in Vivo, as the antibodies selectively recognise and **bind** unique ligands associated with target cells. (II) are esp. useful for treating and locating malignant tumours.

0/1

Dwg.0/1

ABEQ EP 174853 B UPAB: 930922
An antibody conjugate of the formula (I) wherein Ab is the residue of a Fab' fragment of a monoclonal antibody, said antibody being one which retains **antigen-binding** activity following enzymatic removal of the **Fc** fragment and reductive cleavage of the disulfide bond joining the heavy chains; R is a **divalent** organic linker; and R' is a group which is capable of chelating a radionuclide metal ion.

ABEQ US 4837003 A UPAB: 930922
Antibody conjugate of formula (I) is new. In the formula Ab is the residue of a Fab' fragment of monoclonal antibody. The antibody retains **antigen-binding** activity after enzymatic removal of the **Fc**. fragment and reductive cleavage of the disulphide bond joining the heavy chains. S is the residue of the free sulphydryl gp. of the Fab' fragment. R is a **divalent**

organic linker. R' is a nontoxic, weakly acidic chelating gp. which can form a chelate with a radionuclide metal ion that is thermodynamically stable under physiological conditions and has higher stability constant than a chelate of the radionuclide with transferring in vivo.

USE - For detecting localising and treating a tumour in vivo.

L178 ANSWER 24 OF 49 BIOSIS COPYRIGHT 1996 BIOSIS DUPLICATE 12
AN 87:82093 BIOSIS
DN BA83:40671
TI PROPERTIES AND DISTRIBUTION OF A LECTIN-LIKE HEMAGGLUTININ DIFFERENTIALLY EXPRESSED BY MURINE STROMAL TISSUE MACROPHAGES.
AU CROCKER P R; GORDON S
CS SIR WILLIAM DUNN SCH. PATHOLOGY, UNIV. OXFORD, SOUTH PARKS RD., OXFORD 0X1 3RE, UK.
SO J EXP MED 164 (6). 1986. 1862-1875. CODEN: JEMEAV ISSN: 0022-1007
LA English
AB We describe a novel hemagglutinin which is differentially expressed on murine stromal tissue macrophages. Resident bone marrow macrophages (RBMM), which are physically associated with immature, proliferating hematopoietic cells in vivo, formed striking rosettes with unopsonized sheep erythrocytes (E) in vitro, unlike resident peritoneal macrophges (RPM). **Binding** of E was macrophage (M.vphi.) specific, not accompanied by ingestion and independent of temperature (0-37.degree. C), **divalent** cations, and the metabolic inhibitors azide and iodoacetate. Pretreatment of RBMM with trypsin prevented rosette formation, but neuraminidase enhanced it. Conversely, **binding** was virtually aborgated if E were pretreated with neuraminidase, whereas trypsin pretreatment of the ligand resulted in a slight enhancement. The lectin-like nature of the E receptor (SER), with specificity for sialylated glycoconjugates, was consistent with the inhibition of **binding** we saw with neuraminyllactose or the ganglioside GD1a (50% inhibition at 5-10 mM and 11 .mu.M, respectively). Expression of SER on freshly isolated RBMM was heterogeneous and exhibited a striking inverse correlation with expression of Ia **antigens**. During cultivation in 10% **FCS**, levels of SER on RBMM declined with a half-life of .apprx. 24 h. Other cell surface changes induced by cultivation included a transient increase in expression of Ia **antigen** and acquisition of Mac-1. To determine whether SER was expressed on other stromal M.vphi. populations, adherent cells were isolated from various tissues by collagenase digestion or lavage. **Binding** of E was highest on RBMM and lymph node stromal M.vphi., at intermediate levels on Kupffer cells and splenic stromal M.vphi., but was low or undetectable on blood monocytes and thymic, peritoneal, pleural, and bronchoalveolar M.vphi.. SER therefore appeared to be expressed on certain M.vphi. populations embedded in solid tissues but was largely absent from M.vphi. recoverable by lavage. Its absence from monocytes implies that SER is acquired by M.vphi. after entering tissues where it may perform adhesive functions. In bone marrow, SER on RBMM could interact with an appropriate sialylated ligand on murine hematopoietic cells, and could influence their rate of growth and differentiation.

L178 ANSWER 25 OF 49 BIOSIS COPYRIGHT 1996 BIOSIS
AN 86:339644 BIOSIS
DN BA82:53848
TI PROTECTIVE EFFICACY OF MAREK'S DISEASE CVI-988 CEF-65 CLONE C AGAINST CHALLENGE INFECTION WITH THREE VERY VIRULENT MAREK'S DISEASE VIRUS STRAINS.
AU DE BOER G F; GROENENDAL J E; BOERRIGTER H M; KOK G L; POL J M A
CS CENTRAL VETERINARY INST., VIROL. DEP., PO BOX 365, 8200 AJ LELYSTAD, THE NETHERLANDS.
SO AVIAN DIS 30 (2). 1986. 276-283. CODEN: AVDIAI ISSN: 0005-2086
LA English
AB Comparative 50% protective dose (PD50) assays were performed using a plaque-purified preparation of Marek's disease virus (MDV) strain CVI-988 at the 65th chicken embryo fibroblast (CEF) passage level (MDV CVI-988 CEF65 clone C) and three commercial MD vaccines: herpesvirus of turkeys (HVT) **FC126**, MDV CVI-988 CEF35, and a **bivalent** vaccine composed of HVT **FC126** and MDV SB-1. In addition, comparative PD50 assays were performed in groups of chickens with maternal antibody to each of the three vaccines. Three representatives of the newly emerged biovariant very virulent (vv) MDV strains-RB/1B, Tun, and Md5-were employed as challenge virus. The experiments made feasible the differentiation between virulent MDV and vvMDV strains, within serotype 1. Vaccination with CVI-988 clone C vaccine resulted in PD50 estimates of about 5 plaque-forming units (PFUs) against challenge infection with each of the three vvMDV strains. The PD50 estimate of CVI-988 clone C vaccine was 12-fold below the PD50 of HVT **FC126**. The protective synergism of **bivalent** vaccine, composed of HVT and SB-1, was confirmed by groups given the lowest vaccine doses. The **bivalent** vaccine, however, resulted in incomplete protection in groups given the highest vaccine doses. Homologous maternal antibodies to serotype 1 caused a fivefold increase in the PD50 estimate of CVI-988 clone C. Heterologous maternal antibodies against HVT did not interfere with efficacy of CVI-988 clone C vaccination. However, the combination of maternal antibodies against both HVT and SB-1 (serotypes 2 and 3) showed a strong adverse effect on CVI-988 clone C vaccine. The effect of materal antibody induced by **bivalent** vaccine can be explained by the **binding** capacity of antibodies directed to serotype-common **antigenic** determinants of serotype 1 strains, which are partly shared by serotype 2 and which for another part are present on serotype 3 viruses. Results indicate that the mechanism of protective synergism of **bivalent** MD vaccines is mediated by the immune response to the combination of serotype-common **antigenic** determinants of virus serotypes 2 and 3.

L178 ANSWER 26 OF 49 BIOSIS COPYRIGHT 1996 BIOSIS DUPLICATE 13
AN 86:339742 BIOSIS
DN BA82:53946
TI FC FC INTERACTIONS REVEALED BY SPIN-LABELED IMMUNOGLOBULIN G HETEROSACCHARIDES IN MODEL IMMUNE COMPLEXES.
AU KUSUMI A; WINKELHAKE J L

CS MICROPHOTONIC CENT., MED. COLL. WIS., 8701 WATERTOWN PLANK RD., MILWAUKEE, WIS. 53226.
SO BIOCHEM BIOPHYS RES COMMUN 137 (1). 1986. 237-243. CODEN: BBRCA9 ISSN: 0006-291X
LA English
AB Dynamic properties of spin-labelled heterosaccharides in the **Fc**-region of murine monoclonal antihapten immunoglobulin G were studied in model immune complexes (IC) as a function of the IC size. Model IC dimers, trimers and oligomers were formed using **bivalent** photoaffinity **antigens**. The ESR spectrum exhibits two components. The rotational correlation time of the less-immobilized species is shorter than 10-10 sec, and that of the more-immobilized component is in the order to 10-9 .apprx. 10-8 sec depending on the IC size. Fraction of the more-immobilized spin labels increases, and the mobility of this component decreases with increase in IC size (i.e., mobility: monomers .apprxeq. dimers > trimers .mchgt. immune-complex precipitates). These data strongly suggest the existence of **Fc:Fc** interactions in IC, and provide the basis for a model in which such interactions underlie the initial mechanism by which the information of **antigen binding** to Fab region is transferred into organized **Fc:Fc** association structure for IgG effector activities.

L178 ANSWER 27 OF 49 LIFESCI COPYRIGHT 1996 CSA
AN 86:45956 LIFESCI
TI Preparation of F(ab') sub(2) fragments from mouse IgG of various subclasses.
IMMUNOCHEMICAL TECHNIQUES. PART I. HYBRIDOMA TECHNOLOGY AND MONOCLONAL ANTIBODIES.
AU Lamoyi, E.; Langone, J.L. [editor]; van Vunakis, H. [editor]
CS Lab. Immunol., Natl. Inst. Allergy and Infect. Dis., Natl. Inst. Health, Bethesda, MD 20892, USA
SO METHODS ENZYMOL., (1986) pp. 652-662.
DT Book
FS F; W; L
LA English
AB Mouse monoclonal antibodies (MAbs) of defined specificity and immunoglobulin subclass are being applied in vitro in a variety of immunochemical assays and in vivo as diagnostic and therapeutic agents. For some of these purposes it is often advantageous to use antibody fragments that are **bivalent** (i.e., retain both **antigen-binding** sites), but lack the **Fc** region of the IgG molecule. Such F(ab') sub(2) fragments are valuable in serological studies of membrane **antigens** of lymphoid and reticuloendothelial cells which can **bind** IgG "nonspecifically" through the **Fc** receptors in their surfaces. In this chapter, procedures are described in detail for the preparation, assay, and purification of F(ab') sub(2) from the various subclasses of mouse IgG.

L178 ANSWER 28 OF 49 BIOSIS COPYRIGHT 1996 BIOSIS DUPLICATE 14
AN 85:397964 BIOSIS

DN BA80:67956
TI ACTIVATION OF COMPLEMENT PATHWAYS BY UNIVALENT ANTIBODY DERIVATIVES WITH INTACT FC ZONES.
AU WATTS H F; ANDERSON V A; COLE V M; STEVENSON G T
CS LYMPHOMA RESEARCH UNIT, TENOVUS LAB., GENERAL HOSPITAL, SOUTHAMPTON SO9 4XY, U.K.
SO MOL IMMUNOL 22 (7). 1985. 803-810. CODEN: MOIMD5 ISSN: 0161-5890
LA English
AB The ability of 2 univalent antibody derivatives to invoke complement-mediated lysis of guinea-pig L2C leukemic lymphocytes was investigated. The derivatives were Fab/c from rabbit IgG antibody, in which only one Fab arm is removed from the parent molecule and FabFc in which Fab'.gamma., from peptic digestion of sheep IgG antibody, is disulfide-bonded to the **Fc**.gamma. yielded by papain digestion of an arbitrary IgG. Antibody activity was directed against surface IgM on the target cells. Both derivatives could invoke lysis via the classical pathway in the presence of rabbit complement. Exposure of the cells to the derivatives at 37.degree. C before introducing complement yielded no protective **antigenic** modulation. At low complement concentrations the derivatives were more efficient than the parent antibodies at invoking lysis, apparently due to the fact that the derivatives do not cause modulation; it appears that cells can undergo a useful degree of modulation when confronted simultaneously by **bivalent** antibody and low levels of complement. The Fab/c preparations were also able to invoke lysis by guinea-pig complement. Lysis occurred under conditions where activation took place only via the classical pathway (in dilute complement) or only via the alternative pathway (in the absence of Ca ions, or in [complement component] C4-deficient guinea-pig serum). The results demonstrate that there is no need for 2 **antigen-binding** Fab arms in antibody activation of either the classical or alternative complement pathways. They favor models requiring clustering of **Fc** regions rather than steric changes which might follow **binding** of **antigen.**

L178 ANSWER 29 OF 49 BIOSIS COPYRIGHT 1996 BIOSIS DUPLICATE 15
AN 85:312403 BIOSIS
DN BA79:92399
TI A LECTIN-LIKE RECEPTOR ON MURINE MACROPHAGE CELL LINE CELLS MM-1 INVOLVEMENT OF SIALIC-ACID-**BINDING** SITES IN OPSONIN-INDEPENDENT PHAGOCYTOSIS FOR XENOGENEIC RED CELLS.
AU KYOIZUMI S; MASUDA T
CS INSTITUTE IMMUNOLOGY, FACULTY MEDICINE, KYOTO UNIVERSITY, SAKYO, KYOTO, 606 JAPAN.
SO J LEUKOCYTE BIOL 37 (3). 1985. 289-304. CODEN: JLBIE7 ISSN: 0741-5400
LA English
AB The recognition mechanism of xenogenic red cells by mouse macrophages was studied by using established cell lines. Approximately 30% of cell line cells Mm1 which lack La **antig n**, as well as of thioglycolate-induced peritoneal macrophages from SL/Am mice (TGC-M.vphi.) could ingest unopsonized quail red cells (QRC). In

contrast, an undifferentiated type of cell line, M1-, and another type of macrophage cell line, Mk1-C, possessing accessory cell activity in association with the expression of La **antigen**, had no phagocytic activity for QRC. Approximately 80% of Mm1 cells, as well as TGC-M.vphi. formed rosettes with QRC, whereas M1- and Mk1-C cells did not; indicating that specific **binding** sites for QRC are expressed on a large portion of Mm1 and TGC-M.vphi. but not on M1- and Mk1-C cells. No requirement of **divalent** cation (Mg2+, Ca2+) and metabolic energy was observed for rosette formation between Mm1 cells and QRC. Protease treatment of Mm1 cells eliminated the rosette activity, whereas periodate oxidation or glycosidase treatment slightly enhanced this activity, suggesting the involvement of surface protein in **binding** sites of Mm1 cells. In contrast to these findings of Mm1 cells, **binding** components of QRC were sensitive to periodate oxidation or neuraminidase treatment but resistant to protease, suggesting that the terminal sialic acid residues of carbohydrate of QRC are recognized by Mm1 cells. N-acetylneuraminic acid (NeuNAc) inhibited the rosette formation and promoted the dissocation of rosettes already formed. N-Acetylneuramin lactose (NeuNAc-Lact) was more efficient in rosette inhibition than NeuNAc. These sugars also blocked the phagocytosis of QRC by Mm1 cells but had not effect on either **Fc**-mediated phagocytosis or latex ingestion. Phagocytosis of QRC by murine macrophages apparently is mediated by protease-sensitive **binding** sites recognizing terminal sialic acid residues of QRC in conjunction with additional carbohydrates.

L178 ANSWER 30 OF 49 BIOSIS COPYRIGHT 1996 BIOSIS DUPLICATE 16
AN 85:388497 BIOSIS
DN BA80:58489
TI INFLUENCE OF DIFFERENT IMMUNOGLOBULIN G PREPARATIONS ON PHAGOCYTOSIS OF PSEUDOMONAS-AERUGINOSA BY POLYMORPHONUCLEAR GRANULOCYTES.
AU TRAUTMANN M; TRIEST K; HOFSTAETTER T; SEILER F R; HAHN H
CS INST. MIKROBIOL. FREIE UNIV. BERLIN, HINDENBURGDAMM 27, D-1000 BERLIN 45.
SO ZENTRALBL BAKTERIOL MIKROBIOL HYG SER A 259 (1). 1985. 104-117. CODEN: ZBMPEJ
LA English
AB Commercially available Ig products suitable for i.v. application in humans are made by enzymatic or chemical modification of the IgG molecule. In order to examine to which extent in vitro biologic functions of the IgG molecule are preservedin such preparations, specific antipseudomonal IgG from the rabbit was modified according to some of these procedures. The opsonizing activity of the different IgG preparations was evaluated in an in vitro system measuring the phagocytosis of P. aeruginosa by rabbit granulocytes. The results demonstrated that the product Fab/**Fc**, which is made by papain or plasmin degradation of the IgG molecule,was still able to enhance phagocytosis but not to activate complement. The smaller papain- or plasmin-derived fragments Fab and **Fc** had no opsonizing activity. The pepsin-derived product F(ab')2, which possesses a **divalent antigen binding**

site but lacks the **Fc** part, only enhanced phagocytosis when complement concentrations of > 20% were present in the phagocytic system. Since the F(ab')2 fragment is not able to interact with **Fc** receptors or to activate complement via the classical pathway, the phagocytosis-enhancing activity of this molecule must be attributed to alternative complement pathway activation. In contrast to the enzymatically derived IgG preparations, a newly developed S-sulfonated IgG product wasas efficient as unmodified IgG both in **Fc**- and complement-mediated opsonization.

L178 ANSWER 31 OF 49 BIOSIS COPYRIGHT 1996 BIOSIS DUPLICATE 17
AN 85:278987 BIOSIS
DN BA79:58983
TI REGULATION OF T-CELL FUNCTION BY ANTIBODIES ENHANCEMENT OF THE RESPONSE OF HUMAN T-CELL CLONES TO HEPATITIS B SURFACE ANTIGEN BY ANTIGEN-SPECIFIC MONOCLONAL ANTIBODIES.
AU CELIS E; ZURAWSKI V R JR; CHANG T W
CS CENTOCOR, 244 GREAT VALLEY PARKWAY, MALVERN, PA. 19355.
SO PROC NATL ACAD SCI U S A 81 (21). 1984. 6846-6850. CODEN: PNASA6 ISSN: 0027-8424
LA English
AB Eight mouse monoclonal antibodies specific for hepatitis B surface **antigen** (HBsAg) were examined for their effects on the **antigen**-induced proliferative response and lymphokine production of human HBsAg-specific T-cell clones in vitro. While all specifically enhanced the T-cell proliferative response, antibodies of the IgG class were generally more effective than those of the IgM class. Both the **divalent** F(ab')2 and the monovalent Fab fragments of an IgG monoclonal antibody had no effects, indicating that the **Fc** portion of the antibody molecules was required. Since **antigen**-presenting cells bear surface receptors for the **Fc** of IgG and fewer or none for that of IgM, the above results also suggest that antibodies enhance the capture of **antigens** by **antigen**-presenting cells as a result of the **binding** of **antigen**-antibody complexes to the **Fc** receptors on these cells. In addition to potentiating the proliferation of the T-cell clones, antibodies also increased the **antigen**-induced production of interferon-.gamma. by these cells. The present in vitro studies suggest that antibodies may regulate immune response and do so by enhancing **antigen** presentation and thus augmenting **antigen**-induced activation and clonal expansion of T cells.

L178 ANSWER 32 OF 49 BIOSIS COPYRIGHT 1996 BIOSIS DUPLICATE 18
AN 84:234834 BIOSIS
DN BA77:67818
TI SUPPRESSION OF IN-VITRO MONO CLONAL HUMAN RHEUMATOID FACTOR SYNTHESIS BY ANTI IDIOTYPIC ANTIBODY TARGET CELLS AND MOLECULAR REQUIREMENTS.
AU KOOPMAN W J; SCHROHENLOHER R E; BARTON J C; GREENLEAF E C
CS DEP. OF MED., DIV. OF CLINICAL IMMUNOL., UNIV. OF ALABAMA SCH. OF MED., UNIV. OF ALA. IN BRIMINGHAM, BIRMINGHAM, ALA. 35294.
SO J CLIN INVEST 72 (4). 1983. 1410-1419. CODEN: JCINAO ISSN: 0021-9738
LA English

AB Antiidiotypic antibody can modulate expression of idiotype both in vivo and in vitro. Although the precise mechanisms underlying modulation of idiotype expression by antiidiotype remains unclear, a requirement for intact IgG antiidiotypic antibody has been suggested and T cells appear to play a role in some systems. Peripheral blood mononuclear leukocytes (MNL) from a patient with a B cell lymphoma and a circulating IgM.kappa. rheumatoid factor (RF) paraprotein were studied in an effort to delineate mechanisms involved in regulation of idiotype expression by antiidiotypic antibody. One to ten percent of MNL from this patient could be cytoplasmically stained with specific F(ab')2 antiidiotypic antibody. MNL from the patient spontaneously synthesized IgM RF in culture that possessed the same idiotype as the circulating IgM RF paraprotein. Production of RF by MNL was suppressed by pretreatment with either intact IgG or the F(ab')2 fragments of antiidiotypic antibody (50% inhibitory concentration was 0.2 and 1.1 .mu.g/culture, respectively). The Fab' fragment of antiidiotypic antibody was not inhibitory (up to 57 .mu.g/culture) despite retaining demonstrable antiidiotype activity. Suppression of RF production was not observed over the same concentration range with the IgG or F(ab')2 fractions of a non-cross-reactive antiidiotypic antibody prepared against another monoclonal IgM.kappa. RF paraprotein or with IgG or F(ab')2 fractions prepared from normal rabbit serum. Inhibition of RF production by antiidiotypic antibody did not require T cells. Antiidiotypic antibody decreased intracellular and extracellular levels of idiotype indicating diminished synthesis of idiotype by the patient's B cells. Synthesis of IgM RF by MNL obtained from unrelated donors was not suppressed by the antiidiotypic antibody specific for the patient's paraprotein. Antiidiotypic antibody is capable of directly suppressing human B cell release of idiotype. The **bivalent antigen-binding** fragment (F[ab]2) of antiidiotypic antibody is sufficient for mediating such suppression. An intact **Fc** portion of antiidiotypic antibody enhances suppression of idiotype and antiidiotypic antibody inhibits idiotype expression by suppressing synthesis of idiotype.

L178 ANSWER 33 OF 49 BIOSIS COPYRIGHT 1996 BIOSIS DUPLICATE 19
AN 84:299182 BIOSIS
DN BA78:35662
TI THE **BINDING** OF MONO CLONAL ANTIBODIES TO CELL SURFACE MOLECULES A QUANTITATIVE ANALYSIS WITH IMMUNO GLOBULIN G AGAINST 2 ALLO ANTIGENIC DETERMINANTS OF THE HUMAN TRANSPLANTATION ANTIGEN HLA-A-2.
AU WAYS J P; PARHAM P
CS DEPARTMENT OF STRUCTURAL BIOLOGY, STANFORD UNIVERSITY SCHOOL OF MEDICINE, STANFORD, CALIF. 94305, USA.
SO BIOCHEM J 216 (2). 1983. 423-432. CODEN: BIJOAK ISSN: 0306-3275
LA English
AB Monoclonal IgG1 PA2.1 and MA2.1 antibodies recognize polymorphic sites of the human transplantation **antigen** HLA-A2. They are distinguishable because MA2.1 **binds** HLA-A2 and HLA-B17 while PA2.1 **binds** HLA-A2 and HLA-A28*. The affinities of PA2.1-Fab for HLA-A2, 3 HLA-A2 variants and HLA-A28* are similar and

relatively low (1.9 .times. 107 M-1). The affinities of MA2.1-Fab for HLA-A2, 3 HLA-A2 variants and HLA-B17 are similar and high (1.2 .times. 109 M-1). The difference in affinity is due to the rates of dissociation, which give dissociation half-times of 290 min for MA2.1-Fab and 4 min for PA2.1-Fab. For both Fab, equilibrium measurements and kinetic determinations gave consistent estimates for affinity. When PA2.1-F(ab)2 or IgG is incubated with cells it reaches equilibrium within 3 h with most molecules **bound** **bivalently** to the cell. Under similar conditions, MA2.1-F(ab)2 does not reach equilibrium and a significant proportion of moleucles **bound** with 1 and 2 sites are found. For the lower-affinity antibody (PA2.1), estimates of the **binding** constant for 1- and 2-site interactions could be made. By simple Scatchard analysis the avidity of F(ab)2 or IgG is 1.3 .times. 109 M-1, giving an enhancement factor of 68 between **bivalent** and univalent **binding**. This is a measure of the equilibrium constant for the interchange between **bivalent** and univalent **binding**. Analysis of the results with more realistic models indicates that the actual value is larger (103-104 M-1) than 68 M-1. The avidities of F(ab)2 and IgG for HLA-A2 are identical, showing the **Fc** does not interfere with **bivalent** **binding** to cells.

L178 ANSWER 34 OF 49 BIOSIS COPYRIGHT 1996 BIOSIS DUPLICATE 20
AN 83:319677 BIOSIS
DN BA76:77169
TI PROPERTIES OF 2 MONO CLONAL ANTIBODIES DIRECTED AGAINST THE FC AND FAB' REGIONS OF RAT IMMUNO GLOBULIN E.
AU CONRAD D H; STUDER E; GERVASONI J; MOHANAKUMAR T
CS SUBDEP. IMMUNOL., GOOD SAMARITAN HOSP., 5601 LOCH RAVEN BLVD., BALTIMORE, MD. 21239.
SO INT ARCH ALLERGY APPL IMMUNOL 70 (4). 1983. 352-360. CODEN: IAAAAM ISSN: 0020-5915
LA English
AB Hybridoma antibodies directed against the **Fc** and Fab portions of rat IgE were produced by immunizing BALB/c mice with rat IgE and fusing the spleen cells with the nonsecreting plasmacytoma P3/X63Ag8.653. Two of the antibodies, designated as A2 and B5, were extensively characterized. Competitive **binding** experiments using rat IgE from the IR 162 and IR 2 immunocytomas and rat IgG indicated that both A2 and B5 were .epsilon.-chain specific and not anti-idiotype. A2 also exhibited some cross-reactivity with mouse IgE. When IgE was treated with chymotrypsin so as to produce both F(ab')2 and Fab fragments, the enzyme-treated IgE retained reactivity with B5, but the reactivity to A2 was lost. Heat denaturation of IgE at 56.degree. C resulted in a progressive loss of reactivity of the IgE for both A2 and the **Fc** receptor on rat basophilic leukemia cells; the reactivity of B5 remained unchanged. A2 does not evidently interact with the same site on the **Fc** of IgE that is involved in **binding** to the rat basophilic leukemia cell **Fc** receptor; A2 exerted little influence on the **binding** of IgE to rat basophilic leukemia cells. The **antigenic** site for B5 is in the Fab region of the IgE

molecule and A2 reacts with the IgE **Fc**. Use of these antibodies to measure cell-**bound** IgE was also evaluated in dual label experiments, and potential problems in using **divalent** antibodies to quantitate cell surface **antigens** are discussed.

L178 ANSWER 35 OF 49 DISSABS COPYRIGHT 1996 UMI
AN 82:12849 DISSABS Order Number: AAR8223788
TI THE CELL SURFACE RECEPTOR FOR IMMUNOGLOBULIN-E: CHARACTERIZATION OF THE RECEPTOR FROM HUMAN BASOPHILS, RAT MAST CELLS AND RAT BASOPHILIC LEUKEMIA CELLS
AU HEMPSTEAD, BARBARA LOUISE [PH.D.]
CS WASHINGTON UNIVERSITY (0252)
SO Dissertation Abstracts International, (1982) Vol. 43, No. 5B, p. 1338. Order No.: AAR8223788. 141 pages.
DT Dissertation
FS DAI
LA English
AB Mast cells and basophils bear on their cell surfaces receptors which are specific for immunoglobulin E (IgE). The interaction of receptor-**bound** IgE molecules with multivalent **antigen** initiates the noncytotoxic release of histamine and other mediators of immediate hypersensitivity. Apparently, the cross-linking of adjacent receptors is the necessary and sufficient signal for this biological response.

In this study, IgE receptors were isolated from human basophils, rat mast cells and rat basophilic leukemia cells (RBL-1), by repetitive affinity chromatography on IgE-Sepharose. The IgE was radiolabeled and the purified receptor retained the ability to specifically rebind to its ligand IgE via the **Fc** portion of the molecule. The IgE receptor isolated from human basophils appeared very similar to the more extensively characterized RBL-1 cell receptor as both receptors migrated as heterogeneous bands of 45,000 to 65,000 M(,r).

The source of molecular weight heterogeneity of the glycoprotein receptor was determined to be due primarily to the addition of N-linked oligosaccharide chains. Receptor isolated from cells incubated with tunicamycin, an antibiotic which inhibits the addition of oligosaccharide chains to nascent polypeptides, appeared as a discrete band of 38,000 M(,r). This carbohydrate-deficient receptor was able to **bind** IgE, and interact with a 30,000 M(,r) protein which was tightly associated with the IgE receptor. This 30,000 M(,r) protein was not a glycoprotein, but was associated with the IgE receptor in both RBL-1 cells and normal mast cells. Tryptic peptide mapping of the IgE receptor and the receptor-associated protein using reverse phase high pressure liquid chromatography was performed and the dissimilarity of peptide maps of these two proteins strongly suggests that they were structurally different proteins.

Lastly, the phosphorylation of the IgE receptor was investigated. Using intact rat mast cells the IgE receptor was shown to be phosphorylated following stimulation of the cells with the **divalent** cation ionophore A23187. The receptor-phosphate

bond was identified as phosphoserine, and limited time course analysis demonstrated specific phosphorylation of the receptor within 15 seconds of cell stimulation. Since mast cell activation and the coupled release reaction are rapid events occurring within 15 to 45 second of the addition of the stimulating agent, the increase in receptor phosphorylation may be part of the secretory stimulus.

L178 ANSWER 36 OF 49 BIOSIS COPYRIGHT 1996 BIOSIS DUPLICATE 21
AN 82:176589 BIOSIS
DN BA73:36573
TI THE EFFECT OF IMMUNO GLOBULIN G AND IMMUNO GLOBULIN G FRAGMENTS ON THE ABSORPTION OF INHALED ANTIGENS ACROSS THE AIR BLOOD BARRIER OF ISOLATED PERFUSED RABBIT LUNGS.
AU GEOGHEGAN W D; DAWSON C A; CALVANICO N J
CS RESEARCH SERVICE/151, VETERANS ADMINISTRATION MEDICAL CENTER, WOOD, WIS. 53193, USA.
SO IMMUNOLOGY 44 (2). 1981. 331-338. CODEN: IMMUAM ISSN: 0019-2805
LA English
AB Absorption of inhaled soluble protein **antigens** across the alveolocapillary membrane can be inhibited by passive immunization in isolated rabbit lungs. A study was carried out to determine the Ig class and structural features (**Fc**-receptor **binding** , **divalent antigen binding**) required to effect the inhibition. Isolated rabbit lungs from unimmunized rabbits were perfused with autologous blood to which whole antiserum, IgG or IgG fragments specific for either ovalbumin (OA) or human serum albumin (HSA) was added. The lungs were insufflated with an aerosol containing 125I-OA and 131I-HSA and blood samples were analyzed for 125I and 131I in trichloroacetic acid (TCA)-precipitable and TCA-soluble forms for 4 h after insufflation. Whole antiserum and the IgG immunoglobulin fraction of the whole antiserum were equally effective in inhibiting the **antigen** absorption, indicating that the IgG antibody is sufficient for the effect. The F(ab')2 and Fab' fragments of the IgG molecule were as effective as native IgG, indicating that the **antigen-binding** site is the only structural requirement and that **Fc**-receptor and **divalent antigen binding** are not required.

L178 ANSWER 37 OF 49 CANCERLIT
AN 81613430 CANCERLIT
TI MONOCLONAL ANTI-FC RECEPTOR IGG BLOCKS ANTIBODY ENHANCEMENT OF VIRAL REPLICATION IN MACROPHAGES.
AU Peiris J S; Gordon S; Unkeless J C; Porterfield J S
CS Sir William Dunn Sch. Pathology, Univ. Oxford, Oxford, OX1 3RE, England
SO Nature, (1981). Vol. 289, No. 5794, pp. 189-191. ISSN: 0028-0836.
DT Journal; Article; (JOURNAL ARTICLE)
FS ICDB
LA English
EM 8105

AB Antibody-dependent enhancement (ADE) of viral replication was studied using West Nile virus (WNV) replication in cultured P388 D1 mouse macrophages. The ADE of viral replication was quantified with an infectious center assay. Ascites fluids of clones producing anti-WNV antibodies of immunoglobulin subclasses IgG1 (F7/101) and IgG2a (F6/16A), together with anti-mouse macrophage **Fc** receptor (**FcR**) monoclonal IgG antibody and its Fab fragment, were prepared and were used in the study as follows. When the P388 D1 cells (2 x 10(6)) were infected with WNV and incubated with a subneutralizing concentration of anti-WNV antibody F7/101, a 189-fold enhancement in the number of infectious centers was observed. Pretreatment of the cells with anti-**FcR** IgG antibody (240 ug/ml, 40 min, 4 C) blocked this enhancement. The anti-**FcR** IgG antibody also blocked the ADE of viral replication induced by the anti-WNV antibody F6/16A. The Fab fragment of the anti-**FcR** antibody showed less marked inhibition of ADE of viral replication; this inhibition by anti-**FcR** Fab demonstrated, however, that the inhibitory effect of the intact anti-**FcR** IgG on ADE was due to the antibody combining site and not to the **Fc** domain. The reduced inhibitory effect of the Fab fragment was suggested to be due to reversible **binding** to the macrophage **FcR**. The responsibility of the **Fc** fragment of the antiviral antibody for the ADE was confirmed in experiments performed with **bivalent** F(ab')2 pepsin fragments of anti-WNV rabbit antibodies; the F(ab')2 antibody lost its ability to enhance viral replication in P388 D1 cells but retained substantial neutralizing activity and ability to **bind** virus **antigens** on radioimmune **binding** assay. These results demonstrated that ADE of replication can be blocked by a monoclonal antibody specific for the **Fc** receptor. (13 Refs)

L178 ANSWER 38 OF 49 LIFESCI COPYRIGHT 1996 CSA
AN 81:8066 LIFESCI
TI Site of Alkaline Phosphatase Attachment in Alkaline Phosphatase-Immunoglobulin G Complexes.
AU Crofton, P.M.
CS Dept. Biol., Mahidol Univ., Rama 6 Road, Bangkok 4, Thailand
SO CLIN. CHIM. ACTA., (1981) vol. 112, no. 1, pp. 33-42.
DT Journal
FS L
LA English
SL English
AB Sera containing the rare alkaline phosphatase-immunoglobulin G complex were studied to try to determine the type of interaction involved. Pepsin and papain digestion of immunoglobulin G showed that alkaline phosphatase was attached to the F(ab') sub(2) region of the immunoglobulin molecule and not to the **Fc** region. Sialic acid did not play a role in this attachment. Attempts to generate the complex in vitro) using polyclonal immunoglobulin, and attempts to dissociate the complex in vitro , were both unsuccessful. It is concluded that the complex is an immune complex formed by antibody-**antigen** reaction in the circulation,

and consists of two molecules of monovalent alkaline phosphatase associated with one molecule of **divalent** immunoglobulin G.

L178 ANSWER 39 OF 49 BIOSIS COPYRIGHT 1996 BIOSIS DUPLICATE 22
AN 81:195734 BIOSIS
DN BA71:65726
TI THEORY OF CLUSTERING OF CELL SURFACE RECEPTORS BY LIGANDS OF ARBITRARY VALENCE DEPENDENCE OF DOSE RESPONSE PATTERNS ON A COARSE CLUSTER CHARACTERISTIC.
AU DELISI C
CS LAB. THEORETICAL BIOL., NATIONAL CANCER INST., NATIONAL INST. HEALTH, BETHESDA, MD 20205.
SO MATH BIOSCI 52 (3-4). 1980 (RECD. 1981). 159-184. CODEN: MABIAR
ISSN: 0025-5564
LA English
AB The interaction of ligands with cell surface receptors often induces the formation of receptor clusters on the plasma membrane as in fibroblast mitogenesis induced by epidermal growth factor and insulin induced glucose oxidation by adipocytes. Methods for calculating the kinetics of clustering of **divalent** receptors by ligands of arbitrary valence are developed. Intramolecular bonding (i.e., both sites on the same receptor **bound** to a single ligand) is explicitly included in the formalism. The equations are necessary for a quantitative analysis of the response of basophils and mast cells to multivalent haptens and **antigens**. The implications of the equations are briefly pursued. The presence of intramolecular reaction has 2 opposing effects on the concentration of cross-links, increasing them at low ligand concentrations and suppressing them at high ligand concentrations. Those **bivalent** haptens which do not induce a response may be active when they are in the form of higher valence analogs. The relation K = 1/2c* between the ligand concentration at which histamine release peaks (c*) and the ligand site-receptor site affinity (K), which holds for symmetric **bivalent** haptens, has no simple exact analog for multivalent ligand. A good approximate relation that holds when .**GRAPHIC**. > Kc* is .**GRAPHIC**. = 1/**f**c*, where .**GRAPHIC**. is the intramolecular equilibrium constant at very low ligand concentrations, and f (.gtoreq. 2) is the valence of the ligand. The dose dependence of a response that is proportional to concentration of cross-links is qualitatively different from one that is proportional to the concentration of aggregates. In particular, the concentration of aggregates may be a bimodal function of ligand concentration. The deviations from simple unimodal dose-response patterns that are sometimes observed in histamine release experiments may in part reflect the cell's inability to discriminate between cluster sizes beyond a certain small size. The dose dependence of the number of aggregates per cell shows a switch from unimodal to bimodal as valence is increased. The bimodal pattern is markedly asymmetric, with a high concentration peak that is considerably larger than the low concentration peak. For conditions under which such bimodality is obtained, the addition of monomer will decrease of number of aggregates at very low concentrations, but increase the number over the limb that descends from the low concentration mode. For low

affinity ligands (K = 104 M-1), the low concentration mode will be the only one observed. A response that is a function of the number of aggregates will sometimes be observed to increase at supraoptimal concentrations when monomer is added. The number of aggregates induced by **bivalent** ligands can, for certain limited but relevant concentration and parameter ranges, exceed the number induced by higher valence analogs. The predictions are discussed in terms of available experimental data.

L178 ANSWER 40 OF 49 BIOSIS COPYRIGHT 1996 BIOSIS DUPLICATE 23
AN 80:273474 BIOSIS
DN BA70:65970
TI THE REED STERNBERG CELL LYMPHOCYTE INTERACTION ULTRASTRUCTURE AND CHARACTERISTICS OF **BINDING**.
AU PAYNE S V; NEWELL D G; JONES D B; WRIGHT D H
CS FAC. MED., LEVEL E, S. LAB./PATHOL. BLOCK, SOUTHAMPTON GEN. HOSP., SOUTHAMPTON SO9 4XY, ENGL., UK.
SO AM J PATHOL 100 (1). 1980. 7-24. CODEN: AJPAA4 ISSN: 0002-9440
LA English
AB Autologous T [thymus-derived] lymphocytes form broad, unspecialized, noninvaginating contacts with Reed-Sternberg cells [from patients with Hodgkin's disease] in vitro. These differ from contacts between cytotoxic lymphocytes and their targets, and from the uropodal type of lymphocyte adherence described in many **antigen**- (or mitogen-) dependent systems; they show some resemblance to **antigen**-independent lymphocyte/macrophage contacts. Adherence is not confined to a specific T cell subset; most adherent cells are negative for ANAE [.alpha.-naphthyl acetate esterase], **Fc** .gamma. and **Fc** .mu. receptors. A minority have **Fc** .mu. or **Fc** .gamma. receptors or show ANAE staining. Adherence is dependent on **divalent** cations and intact surface proteins but is independent of temperature, cell metabolism and intact microtubules, and does not appear to be mediated by **Fc** .gamma. receptors or Ig[immunoglobulin]G. These characteristics distinguish it from immune T cell/target-cell **binding**, and from **antigen**-independent T cell/macrophage or T cell/B [bone marrow-derived] lymphoblast **binding**. Contrary to previous suggestions, this interaction is not related to a cytotoxic attack. The lack of similarities with other lymphocyte adherence systems leads to the suggestion that a unique receptor system is involved.

L178 ANSWER 41 OF 49 BIOSIS COPYRIGHT 1996 BIOSIS DUPLICATE 24
AN 79:251351 BIOSIS
DN BA68:53855
TI INTERACTION OF A BIVALENT LIGAND WITH IMMUNO GLOBULIN M ANTI LACTOSE ANTIBODY.
AU KARUSH F; CHAU M-M; RODWELL J D
CS DEP. MICROBIOL., UNIV. PA. SCH. MED., PHILADELPHIA, PA. 19104, USA.
SO BIOCHEMISTRY 18 (11). 1979. 2226-2232. CODEN: BICHAW ISSN: 0006-2960
LA English
AB A model system was developed for the study of the interaction between **bivalent** ligands and multivalent antibody. This system

utilized modified [human] .beta.2-microglobulin as the carrier of the reactive lactosyl groups and equine anti-lactose Ig[immunoglobulin]M antibody. Chemical modification of the carrier allowed conjugation of 2 such groups to cysteinyl residues at positions 25 and 81 with a potential separation of 20 nm. Each lactosyl group contained a 2,4-dinitrophenyl moiety which served as a sensor of the **binding** of the group to an antibody site and permitted **binding** measurements by fluorescence quenching. Examination by analytical ultracentrifugation demonstrated no significant cross-linking by the **bivalent** ligand of either the IgM antibody or the monomer (IgMs) derived from it. **Binding** constants for complex formation between the **bivalent** and monovalent ligands and IgM, IgMs and Fab.mu. were determined by fluorescence titrations. These measurements also established that the **bivalent** ligand was **bound** to IgM and IgMs primarily as a cyclic complex. The small enhancement factor (.apprx. 3-fold) for the **binding** of the **bivalent** ligand compared with the monovalent one was attributed to the loss of configurational entropy associated with the formation of the cyclic complex. The complex of **bivalent** ligand and IgM antibody was effective in the depletion of complement in contrast to the complex with the small monovalent ligand. This **antigen**-mediated effector activity is apparently the consequence of a distortion of the normal planar structure of the IgM molecule. The structural perturbation would consist of a bending of some of the F(ab')2 regions out of the plane containing the (**Fc**.mu.)5 core due to the cross-linking of adjacent F(ab')2 regions by the **bivalent** ligand.

L178 ANSWER 42 OF 49 BIOSIS COPYRIGHT 1996 BIOSIS DUPLICATE 25
AN 80:159468 BIOSIS
DN BA69:34464
TI DE GRANULATION OF HUMAN BASOPHILS QUANTITATIVE ANALYSIS OF HISTAMINE RELEASE AND DE SENSITIZATION DUE TO A BIVALENT PENICILLOYL HAPTEN.
AU DEMBO M; GOLDSTEIN B; SOBOTKA A K; LICHTENSTEIN L M
CS THEOR. DIV., LOS ALAMOS SCI. LAB., UNIV. CALIF., LOS ALAMOS, N.M. 87545, USA.
SO J IMMUNOL 123 (4). 1979. 1864-1872. CODEN: JOIMA3 ISSN: 0022-1767
LA English
AB A quantitative model of histamine release from human basophils due to the symmetric **bivalent** hapten, bis-benzylpenicilloyl 1,6-diaminohexane, (BPO)2 is presented. The major elements of the model are given. The histamine content of basophils is divided into a large number of discrete and quasi-independent quanta. A certain fraction of the quanta in a population of basophils are non-releasable. Release of the contents of a quanta is a stochastic event. The **binding** and cross-linking reaction between (BPO)2 and cell surface Ig[immunoglobulin]E approaches thermal equilibrium on a time scale that is very short compared to the time scale of histamine release from or desensitization of basophils. The probability per unit time of the release event, PR, is a function of the extracellular Ca concentration [Ca]ex, and of the number of cross-linked IgE molecules per cell, Xpoly. The functional dependence

of PR on Xpoly for fixed [Ca]ex is sigmoid, and the dependence of PR on [Ca]ex is such that PR .fwdarw. 0 as [Ca]ex .fwdarw. 0 for all values of Xpoly. **Antigen**-specific IgE molecules and/or their associated **Fc** receptors are inactivated at a rate that is a saturable function of Xpoly. This process of inactivation is not dependent on the extracellular Ca concentration. The model is tested by carrying out a detailed fitting to histamine release data obtained by using cells from a single donor. Good agreement between theory and experiment is obtained, and all the parameters of the model are determined. The ways in which the model can be extended to include systems more complex than the (BPO)2 system are briefly discussed.

L178 ANSWER 43 OF 49 EMBASE
AN 80052390 EMBASE
TI Interactions between Fab and Fc regions in liganded immunoglobulin G. A study employing the antigenic polymorphism and multivalency of the human red-cell membrane.
AU Romans D.G.; Tilley C.A.; Dorrington K.J.
CS Dept. Biochem., Univ. Toronto, Toronto, Ontario, Canada
SO AEROMED. REV., (1978) No. 1 (859-879).
CODEN: AMRWBY
CY United States
LA English
AB Previous studies indicated that the inter-.gamma. chain disuphide bonds restrict segmental flexibility within the **Fc** region of human IgG; the present study suggests that there are also non-covalent hinge-region restrictions of flexibility in the liganded molecule. Staphylococcus aureas protein A-Sepharose-passed F(ab')2 fragments from IgG fractions of blood-group antisera were tested in parallel with the parent antibodies in haemagglutination tests. At molar-equivalence in protein, the titre of F(ab')2 from incomplete antibodies was 2- to 3-fold greater than the titre of the intact antibody ($p<0.001$). Increased activity occurred whether enzyme or albumin techniques were used to convert the incomplete antibodies to agglutinins, and was particularly striking with Kell blood-group antibodies where F(ab')2 fragments were weak saline agglutinins. Antiglobulin tests, using anti-K, revealed small differences in intrinsic **antigen-binding** activity between IgG and F(ab')2 fractions, but these differences were either of inverse relationship or insufficient to account for the increased agglutinating activity of F(ab')2 compared with the whole molecule. By contrast, with IgG saline agglutinins [anti-K(anti-glycophorin A(M)) and anti-B], the apparent freedom introduced by peptic removal of **Fc**, or by reduction of interchain disulphide bonds, was inimical to agglutination, as would be predicted from other studies (Hornick & Karush, 1972; Crothers & Metzger, 1972). These results show that the **Fc** region imposes limitations on full segmental flexibility of Fab regions in liganded IgG. A comparison of the intrinsic and functional **antigen-binding** activities of IgG anti-M and anti-B confirmed that both antibodies engage in substantial monogamous **bivalency**. That the relative monogamous

bivalency of F(ab')2 and IgG anti-M was unaffected by an increased mean separation of M sites on the cell surface suggests that it is predominantly dimers of glycophorin A(M), in situ, which generate the monogamous **bivalency** and that, on M/N red cells, some glycophorin-A(M) molecules are associated as homologous dimers. For IgG anti-A and anti-B, the known decreased affinity with A, and A, B red cells can be explained by the fewer opportunities for monogamous **bivalency**. Using the increased flexibility in F(ab')2 and reduced-alkylated IgG anti-D as molecular probes for spatial relationships between D **antigens**, (i) a marked deviation from an average separation of **antigens** was found on normal red cells under conditions of agglutination, and (ii) an early effect of trypsin-treatment (<2 min) which permits limited redistribution of D sites was detected. Mildly reduced IgG3 exhibited significantly greater flexibility than reduced IgG1, presumably reflecting the extended .gamma.3 hinge region. Unreduced IgG3, however, did not show the increased Fab-region flexibility shown by F(ab')2 fragments on the IgG1 sub-class. In reduced IgG molecules, C.gamma.2 and C.gamma.3 domains retained much of their native surface topography.

L178 ANSWER 44 OF 49 BIOSIS COPYRIGHT 1996 BIOSIS DUPLICATE 26
AN 78:221430 BIOSIS
DN BA66:33927
TI MOBILE FC REGION IN THE ZIE IMMUNO GLOBULIN G-2 CRYO GLOBULIN COMPARISON OF CRYSTALS OF THE FAB-PRIME-2 FRAGMENT AND THE INTACT IMMUNO GLOBULIN.
AU ELY K R; COLMAN P M; ABOLA E E; HESS A C; PEABODY D S; PARR D M; CONNELL G E; LASCHINGER C A; EDMUNDSON A B
CS DEP. BIOCHEM., UNIV. UTAH, SALT LAKE CITY, UTAH 84112, USA.
SO BIOCHEMISTRY 17 (5). 1978 820-823. CODEN: BICHAW ISSN: 0006-2960
LA English
AB A human Ig[immunoglobulin]G2 (.kappa.) Ig (Zie) and the **bivalent antigen-binding** fragment [F(ab')2] derived from it by peptic hydrolysis were crystallized in forms suitable for X-ray diffraction studies. The parent protein and the fragment have cryoglobulin properties. The IgG2 molecule crystallized at 15.degree. C in 0.1 M sodium borate-0.05 M sodium chloride, pH 7.0, and the F(ab')2 fragment crystallized in 0.02 M sodium phosphate, pH 7.4. The crystals were nearly isomorphous. The space group was I222 or I212121, with a = 100.3, b = 153.1, and c = 188.5 .ANG. for the IgG2 protein and a = 99.6, b = 150.7, and c = 192.7 .ANG. for the F(ab')2 fragment. Precession photographs of the hkO, hOl, and Okl projections were markedly similar for the 2 molecules. A comparison of 409 pairs of reflections from the 6.5-.ANG. shells of the 2 crystals indicated a mean difference (RF) in amplitudes of 0.155. The striking similarities indicate that the structural features contributing to the diffraction patterns are nearly the same in the 2 crystals. The Fab' regions have similar conformations in the fragment and parent molecule. The **Fc** region does not contribute significantly to the diffraction pattern of the IgG2 protein. The ability of the **Fc** region to adopt more than 1 conformation with respect to the Fab' arms in the crystal

lattice adds to previous chemical and crystallographic evidence for the biological importance of the flexibility of Ig molecules.

L178 ANSWER 45 OF 49 BIOSIS COPYRIGHT 1996 BIOSIS DUPLICATE 27
AN 77:240446 BIOSIS
DN BA64:62810
TI MITOGENIC PROPERTIES OF FAB AND FAB-PRIME-2 FRAGMENTS OF RABBIT ANTI HUMAN BETA-2 MICRO GLOBULIN FOR HUMAN LYMPHOCYTES.
AU RINGDEN O; JOHANSSON B G
SO SCAND J IMMUNOL 6 (4). 1977 281-289. CODEN: SJIMAX ISSN: 0300-9475
LA Unavailable
AB **Bivalent** F(ab')2 fragments and monovalent Fab fragments of rabbit anti-human .beta.2-microglobulin (anti-.beta.2m) stimulated DNA synthesis in human lymphocytes. Mitogenicity of anti-.beta.2m antibodies can be ascribed to the **antigen-binding** site and not to the **Fc** portion of the molecule. The mitogenic response to F(ab')2, and sometimes Fab, fragments of anti-.beta.2m IgG was comparable to that obtained with the original IgG antibodies when tested at the same protein concentration. Since Fab monomers of anti-.beta.2m can cause lymphocyte activation, cross-linking of hypothetical .beta.2-microglobulin-containing lymphocyte receptors does not seem necessary for activation. F(ab')2 and Fab fragments of anti-.beta.2m blocked the cytotoxic effect of anti-.beta.2m IgG, showing that the fragments did indeed react with .beta.2-microglobulin on the cell surface. F(ab')2 dimers, but not Fab monomers, of anti-.beta.2m were capable of inhibiting the cytotoxic effect of an anti-HLA-A2 antiserum. The mitogenic activity of anti-.beta.2m IgG and Fab monomers of such antibodies disappeared after absorption with highly purified .beta.2-microglobulin. The mitogenic effect of anti-.beta.2m IgG was inhibited to a minor extent by exposure of cells to high concentrations of pooled multispecific anti-HLA antibodies. This effect was probably nonspecific.

L178 ANSWER 46 OF 49 MEDLINE
AN 75134348 MEDLINE
TI Regulation of antibody response in vitro. IX. Induction of secondary anti-hapten IgG antibody response by anti-immunoglobulin and enhancing soluble factor.
AU Kishimoto T; Ishizaka K
SO JOURNAL OF IMMUNOLOGY, (1975 Feb) 114 (2 Pt 1) 585-91. Journal code: IFB. ISSN: 0022-1767.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Abridged Index Medicus Journals; Priority Journals
EM 7508
AB Attempts were made to induce antibody response of hapten-primed cells by stimulation with anti-immunoglobulin (Ig) antibody and cellfree supernatant (CFS) which contained nonspecific enhancing factor. Mesenteric lymph node cells were obtained from rabbits which were primed with dinitrophenylated ragweed **antigen** (DNP-Rag), and the primed cells were incubated in vitro with either anti-rabbit gamma-chain or anti-Fab antibody for 24 hr. After

washing, the cells were cultured in CFS which was obtained from the culture of DNP-Ascaris (DNP-Asc)-primed lymph node cells with free carrier (Asc). The results showed that hapten-primed cells were triggered for IgG anti-DNP antibody response by the two reagents, i.e., anti-Ig antibody and CFS, both which did not share any **antigenic** specificity with the priming **antigen** (DNP-Rag). Neither anti-Ig alone nor CFS alone induced anti-hapten antibody response. Anti-Ig and CFS triggered not only hapten-primed B cells but also the other IgG-bearing (B) cells for IgG synthesis. Lymph node cells of umprimed animals were activated for IgG synthesis by the stimulation with anti-Ig followed by culture in CFS. Evidence was obtained that anti-Ig antibody has to be **divalent** for the activation of B cells. Stimulation of DNP-primed cells with the F(ab')2 fragment of the antibody and CFS-induced anti-hapten antibody response, whereas the Fag' fragment of the anti-Ig plus CFS failed to do so. The results suggested that bridging of cell-surface Ig receptors by multivalent ligand is the initial step of B cell activation. Another evidence for the activation of hapten-primed B cells by anti-Ig was obtained by supplemental immunization of DNP-Asc-primed animals with the **Fc** fragment of goat IgG. Stimulation of mesenteric lymph node cells from these animals with goat anti-rabbit-Ig alone (no CFS) resulted in the formation of anti-DNP IgG antibody. The results indicated that lymphocytes primed for goat IgG collaborated with DNP-primed IgG-B cells when the lymph node cells were stimulated with goat anti-Ig.

L178 ANSWER 47 OF 49 EMBASE
AN 76100459 EMBASE
TI Cell surface IgE on human basophil granulocytes.
AU Ishizaka T.; Ishizaka K.
CS Dept. Med., Johns Hopkins Univ. Sch. Med., Baltimore, Md. 21239, United States
SO ANN.N.Y.ACAD.SCI., (1975) Vol.254 (462-475).
CODEN: ANYAA
LA English
AB Recent knowledge on the kinetics of the **binding** reaction between IgE and receptors on basophils is summarized, and the distribution of cell **bound** IgE and IgE receptors after the **antigen** antibody reaction on the cell surface is demonstrated. The following aspects are dealt with: kinetics of the **binding** reaction between IgE and basophil granulocytes (including tables on the IgE molecules per basophil granulocyte, passive sensitization with IgE, radioautography of basophil granulocytes treated with 125I labeled E myeloma protein, the presence and uptake of IgE by basophils exposed to pH 4.0 and the affinity of cell receptor to IgE); redistribution of cell **bound** IgE and receptor sites on basophil granulocytes; distribution of IgE and receptor after cap formation; and the effect of sensitization with IgE on the redistribution of IgE receptors. The findings are summarized as follows: IgE molecules combine with basophils and mast cells through the **Fc** portion of the molecules. This process, which is responsible for passive

sensitization was analyzed at the cellular level. The number of cell **bound** IgE molecules per human basophil was 10,000-40,000 in both atopic and normal individuals. The number of receptors for IgE on the cell was 30,000-90,000. This **binding** is reversible, and no covalent bond is involved. Upon exposure of basophils to pH 4 or lower, cell **bound** IgE completely dissociated from the receptor. At physiologic conditions, however, IgE molecules avidly **bound** with the receptor. The high association constant of the **binding** reaction is probably responsible for a long persistence of passive sensitization with IgE antibody. The number of IgE molecules on the basophil surface has biologic significance. An inverse relation was observed between the number of cell **bound** IgE molecules and the optimal concentration of anti IgE for maximal histamine release. The results suggested that the number and affinity of cell **bound** IgE antibody determine the sensitivity of the cells to **antigen**, but the extent of histamine release is controlled by enzyme systems in the cells. The basophil membrane appears to be in a liquid state, and surface immunoglobulin is movable at 37.degree.C. When the cell **bound** IgE on basophils reacted with **divalent** anti IgE, the immunoglobulin molecules formed patches and eventually migrated into one pole of the cells. The monovalent, Fab fragment of the antibody combined with the cell **bound** IgE but failed to induce redistribution of the cell **bound** immunoglobulin. This finding is in agreement with the fact that **divalent** anti IgE induced histamine release, whereas the Fab fragment of the antibody failed to do so. Subsequent experiments, however, demonstrated that cap formation of cell **bound** IgE was not involved in the process of histamine release. Upon cap formation by anti IgE, receptor molecules and IgE migrated as a complex. Evidence was obtained that not only IgE **bound** receptors but also free receptors migrated to the same pole upon capping. The results strongly suggest that a group of receptor sites are associated with each other at the cell membrane or that receptor molecules on the basophil membrane are multivalent with respect to the combining sites for IgE.

L178 ANSWER 48 OF 49 EMBASE COPYRIGHT 1996 ELSEVIER SCI. B.V.
AN 74211992 EMBASE
TI In vitro suppression of immunoglobulin allotype synthesis by Fab and F(ab')2 fragments of anti allotype antibody.
AU Schuffler C.; Dray S.
CS Dept. Microbiol., Univ. Illinois Med. Cent., Chicago, Ill. 60612, United States
SO CELL.IMMUNOL., (1974) 11/1-3 (367-376).
CODEN: CLIMB8
LA English
AB The F(ab')2 and Fab fragments were prepared from IgG containing antiallotype antibody (Ab) specific for the kappa light chains (i.e. anti b4) and were tested for their ability to suppress b4 allotype production in vitro. Spleen cells from a b4b5 heterozygous rabbit were incubated for 24 hr with the F(ab')2 or Fab fragments and then washed and cultured for an additional 4 days. The tissue culture

supernatant fluids were analyzed for the b4 and b5 allotypes by a radioimmunoassay. The results clearly indicated that both types of IgG fragments can suppress the production of the b4 allotype. Thus, neither the **Fc** fragment nor the **bivalency** of the intact Ab molecule is essential for suppressing activity. This makes it highly unlikely that the allotype suppression in vitro occurs as a result of cell death. When equivalent amounts of IgG, F(ab')2, or Fab anti b4 preparations, as determined by their **antigen binding** capacity, were compared for their relative efficiencies in the induction of b4 allotype suppression, the IgG and F(ab')2 were found to be equally efficient, while the Fab fragment was less efficient than the **bivalent** Ab preparations.

L178 ANSWER 49 OF 49 CANCERLIT
AN 72702810 CANCERLIT
TI MERCURY DERIVATIVES OF THE FAB AND FC FRAGMENTS OF A HUMAN MYELOMA PROTEIN.
AU Steiner L A; Blumberg P M
CS Dept. Biol., Massachusetts Inst. Tech., Cambridge
SO Biochemistry, (1971). Vol. 10, No. 25, pp. 4725-4739.
ISSN: 0006-2960.
DT Journal; Article; (JOURNAL ARTICLE)
FS CARC
LA English
EM 7512
AB **Divalent** mercury ion (Hg2+), a bifunctional reagent highly specific for sulfhydryl groups, was used to study the role of interchain bridges in the structural organization of IgG1 myeloma protein. The development of a generally applicable method for preparing heavy metal derivatives of immunoglobulins or other proteins for X-ray diffraction was also attempted. Myeloma protein and its Fab and **Fc** fragments were characterized by DEAE-cellulose chromatography, Sephadex G-100 gel filtration, polyacrylamide gel electrophoresis and zone electrophoresis. Hg2+ was **bound** to partially reduced **Fc** fragments with a saturation point of approximately two moles Hg2+/mole **Fc**, with no Hg2+ **binding** to unreduced **Fc** fragments. It was concluded that the **binding** sites were the two disulfide cross bonds of the **Fc** chains, probably with a configuration of the type S-Hg-S. Physical and **antigenic** properties of Hg-**bound** **Fc** were the same as those of native **Fc**. X-ray diffraction analysis of **Fc**-Hg fragments revealed patterns quite similar to those of native **Fc** although the **Fc**-Hg crystals were not isomorphous with native crystal as the disulfide bonds were altered by the Hg2+ atoms. Dissociating solvents reduced the **Fc**-Hg fragments to monomers. Titration of Fab with Hg2+ produced results analogous to those with **Fc** titration, with saturation at 0.8 mole Hg2+/mole Fab. Fab-Hg and native Fab were indistinguishable physically and **antigenically**. SDS-polyacrylamide gel electrophoresis of Fab-Hg showed that the major components corresponded to the monomer or dimer of **Fc**

, to native Fab and to the dimer of the light chain when Fab-Hg was in a dissociating solvent. Exposure of 203Hg2+-protein to either unlabeled Hg2+ or to cysteine could completely dissociate both Fc-Hg and Fab-Hg. The Hg2+-sulfur bond, however, was generally stable to a number of reagents in nondissociating solvents.

FILE 'CA' ENTERED AT 16:14:19 ON 02 APR 96
USE IS SUBJECT TO THE TERMS OF YOUR CUSTOMER AGREEMENT
COPYRIGHT (C) 1996 AMERICAN CHEMICAL SOCIETY (ACS)

FILE 'CAPLUS' ENTERED AT 16:14:19 ON 02 APR 96
USE IS SUBJECT TO THE TERMS OF YOUR CUSTOMER AGREEMENT
COPYRIGHT (C) 1996 AMERICAN CHEMICAL SOCIETY (ACS)

- Author (s)

L57 10 SEA FILE=CA "RING D"/AU OR "RING D F"/AU OR "RING DAVID F "/AU OR "RING DAVID FRANCIS"/AU

=> s l57 not (l21 or l67 or l120)
L179 10 FILE CA
L180 10 FILE CAPLUS

TOTAL FOR ALL FILES
L181 20 L57 NOT (L21 OR L67 OR L120)

=> s l181 and (fc? or cd16 or cd 16)
L182 0 FILE CA
L183 0 FILE CAPLUS

TOTAL FOR ALL FILES
L184 0 L181 AND (FC? OR CD16 OR CD 16)

=> s ran=,1994
ENTER LOGIC EXPRESSION, QUERY NAME, OR (END):l181
L185 8 FILE CA
L186 8 FILE CAPLUS

TOTAL FOR ALL FILES
L187 16 L181

=> dup rem l187
PROCESSING COMPLETED FOR L187
L188 8 DUP REM L187 (8 DUPLICATES REMOVED)

=> d 1-8 .beverly; fil
biosi,medl,embas,lifesci,biotechds,cancerlit,wpids,dissabs,confsci,scisearch

L188 ANSWER 1 OF 8 CA COPYRIGHT 1996 ACS DUPLICATE 1
AN 106:154816 CA
TI Microbial production of polysaccharide pellicles and manufacture of

wound dressings from the polysaccharides
SO Eur. Pat. Appl., 46 pp.
CODEN: EPXXDW
IN **Ring, David Francis**; Nashed, Wilson; Dow, Thurman
AI EP 86-304956 860626
PI EP 206830 A2 861230
PY 1986
AB Fibrous polysaccharide (e.g. cellulose) pellicles are manufd. by cultivation of Acetobater or other microorganisms and processed to obtain laminar polysaccharide structures, which are useful in the manuf. of wound dressings, bandages, packaging and wrapping materials, etc. A. xylinum Was cultivated in a modified Schramm/Hestrin medium at 20.degree. for 7 days to produce pellicles, which were processed by pressing, NaOH treatment and washing to yield a self-supporting laminar polysaccharide structure. The processed pellicles were layered or merged into interconnected, densified lamellae that border or circumscribe zones of relatively low fibril d. A water-loaded lamella was hand-pressed to form a membrane-like sheet having a thickness of .apprx.1 mm and a water-cellulose ratio of .apprx.8:1. The sheet is useful as a protective wound covering or surgical wipe.

L188 ANSWER 2 OF 8 CA COPYRIGHT 1996 ACS DUPLICATE 2
AN 101:149803 CA
TI Liquid loaded pad for medical applications
SO Brit. UK Pat. Appl., 8 pp.
CODEN: BAXXDU
IN **Ring, David Francis**; Nashed, Wilson; Dow, Thurman
AI GB 83-33461 831215
PI GB 2131701 A1 840627
PY 1984
AB A cellulose [9004-34-6] pellicle is produced by fermn. with Acetobacter xylinum which is used as an absorbent pad for surgical dressings. Thus, a tray contg. culture medium to a depth of 1.5 cm was inoculated with 108 A. xylinum cells/mL and held undisturbed at 20.degree. for 9 days. A fibrous pellicle 1.5 cm thick formed. It was pressed dry, treated with NaOH to inactivate entrapped bacteria, neutralized with 3% HCl, and repeatedly rinsed by absorption of water and pressing to expel liq. The pellicle retained a thickness of .apprx.1 cm. It had a cellulose content of 40 g/m2 and, when satd., held 3600 g water/m2. It had far greater absorptive capacity than conventional gauze, with excellent strength and handling characteristics.

L188 ANSWER 3 OF 8 CA COPYRIGHT 1996 ACS DUPLICATE 3
AN 75:140076 CA
TI Reaction of oxygen atoms with cyclopentene
SO J. Phys. Chem. (1971), 75(20), 3056-61
CODEN: JPCHAX
AU Cvetanovic, R. J.; **Ring, D. F.**; Doyle, L. C.
PY 1971
AB Mol. rearrangements and fragmentation subsequent to the primary addn. of the ground-state O atoms, O(3P), at 25, 125, and

175.degree. to the double bond in cyclopentene were studied. The major products are cyclopentene oxide, cyclopentanone, cyclobutylcarboxaldehyde, ethylene, and acrolein. Dihydropyran, 4-pentenal, CO, and two unidentified products (perhaps cis- and trans-2-pentenal) are formed in much smaller amts. The type of products formed and the effect of pressure on their yields are fully consistent with a primary formation in the addn. reaction of a triplet biradical intermediate.

L188 ANSWER 4 OF 8 CA COPYRIGHT 1996 ACS DUPLICATE 4
AN 71:12261 CA
TI Methylene radical reaction with cis-2-butene
SO Int. J. Chem. Kinet. (1969), 1(1), 11-27
CODEN: IJCKBO
AU **Ring, David F.**; Rabinovitch, B. S.
PY 1969
AB A study of the pressure dependence of the C5 products from the reaction of cis-2-butene and methylene is reported. Methylene was produced by the photolysis of CH2N2 with 4358 A. light at 23.degree. or 56.degree., and by photolysis of ketene with 3200 A. radiation at 23.degree. or 100.degree.. The change with increasing pressure of the relative amts. of the characteristically triplet products (trans-1,2-dimeth-ylcyclopropane, trans-2-pentene, and 3-methyl-1-butene and singlet products (cis-1,2-dimethylcyclopropane and cis-2-pentene are discussed. The behavior is reminiscent of that found in 3CH2-cis-2-butene systems can be interpreted in terms of the rapid rate of rearrangement of an initial triplet diradical product component, due to 3CH2, relative to the slower rate and readier collisional stabilization of an initial vibrationally-excited dimethyl-cyclopropane product component, due to 1CH2.

L188 ANSWER 5 OF 8 CA COPYRIGHT 1996 ACS DUPLICATE 5
AN 70:15962 CA
TI Reactions of singlet and triplet methylene
SO (1968) 228 pp. Avail.: 68-12,712
From: Diss. Abstr. B 1968, 29(3), 971
AU **Ring, David F.**
PY 1968
AB Unavailable

L188 ANSWER 6 OF 8 CA COPYRIGHT 1996 ACS DUPLICATE 6
AN 69:58709 CA
TI Reactions of triplet methylene radicals with alkanes
SO Can. J. Chem. (1968), 46(14), 2435-50
CODEN: CJCHAG
AU **Ring, D. F.**; Rabinovitch, B. S.
PY 1968
AB The reaction between triplet methylene and propane, butane, and isobutane was examd. Triplet methylene was produced by photolysis of CH2N2 in excess N. The relative rates of primary/secondary/tertiary H abstraction from C-H bonds are .apprx.1:14:150. The rate of addn. of CH2 to the double bond in ethylene appears to proceed .apprx.3.5 times faster than H

abstraction from a tertiary C-H bond. The possible occurrence of insertion by CH2 into C-H bonds is discussed and relative rates of insertion for primary/secondary/tertiary bonds are deduced to be .apprx.1:2:7. Comparisons of the relative rates of insertion to abstraction are also given. Studies with propane-d6 indicated an isotope effect for abstraction of H:D from the primary position of .apprx.3.9; insertion by CH2 into the same position showed an isotope effect of .apprx.2. 15 references.

L188 ANSWER 7 OF 8 CA COPYRIGHT 1996 ACS DUPLICATE 7
AN 70:25482 CA
TI Multi-stage modular column gas chromatography
SO J. Gas Chromatogr. (1968), 6(10), 531-2
CODEN: JGCRAY
AU Rynbrandt, J. D.; **Ring, D. F.**; Rabinovitch, B. S.
PY 1968
AB Mixts. are resolved by multi-stage modular column chromatog. by using columns connected in series or parallel. Any component or group of components can be directed to addnl. columnar or detection process by means of valves. The method eliminates spreading and tailing. However, some loss of resoln. can result due to manipulation of valves. The method is illustrated by sepn. of 0.25 cc. gas mixt. on 3 columns (0.75, 10, and 25 ft.) packed with 14% Octoil on 60-mesh acid-washed firebrick by using 3-stage He streams at 40 psi. and 40 cc./min. and controlled by 4-way valves. After passage of C2H4 through all columns and isobutane to the 3rd column, streams are crossed at valve 2 and cis-2-butene is vented via stream 3 to detector 2. Propane and isobutane are passed through column 3 to detector 1. The product, 2,4-dimethylhexane, which appears on a small tail of 2,4-dimethylpentane, is retained on column 1 for 22 min. and detected on detector 1 by crossing streams at valve 1 and 3. The parent 2,4-dimethylpentane is vented after passage through column 2 and detected on detector 1 by crossing streams at valves 2 and 3.

L188 ANSWER 8 OF 8 CA COPYRIGHT 1996 ACS DUPLICATE 8
AN 68:38779 CA
TI Triplet methylene radical reaction with cis-but-2-ene
SO J. Phys. Chem. (1968), 72(1), 191-8
CODEN: JPCHAX
AU **Ring, David F.**; Rabinovitch, Benton S.
PY 1968
AB A study of the pressure dependence of the C5 products of the reaction between cis-but-2-ene and triplet methylene radicals is reported. Triplet methylene was produced by collisional deactivation (in the presence of a large excess of N) of the methylene produced by the photolysis of CH2N2 with 4358-A. radiation at 23.degree., and with unfiltered light at 23 and 56.degree.. Reaction pressures were varied from 0.3 to 3.5 atm.; a const. ratio of cis-but-2-ene-CH2N2-N was maintained at 20:1:30,000. The observed pressure dependence of the product proportions indicates that alkene products arise, in part, by isomerization of the triplet dimethylcyclopropanes or diradicals, rather than exclusively by a

concomitant noninterceptible formation reaction. There is a pressure dependence of the ratio of trans-cis-dimethylcyclopropane which, together with the pressure variability of products such as 3-methyl-1-butene, dictates caution in the interpretation of the proportions of 3CH2:1CH2 from product proportions in photolysis systems and provides an alternative interpretation of a previous finding of the authors. 15 references.

FILE 'BIOSIS' ENTERED AT 16:19:13 ON 02 APR 96
COPYRIGHT (C) 1996 BIOSIS(R)

FILE 'MEDLINE' ENTERED AT 16:19:13 ON 02 APR 96

FILE 'EMBASE' ENTERED AT 16:19:13 ON 02 APR 96
COPYRIGHT (C) 1996 Elsevier Science B.V. All rights reserved.

FILE 'LIFESCI' ENTERED AT 16:19:13 ON 02 APR 96
COPYRIGHT (C) 1996 Cambridge Scientific Abstracts (CSA)

FILE 'BIOTECHDS' ENTERED AT 16:19:13 ON 02 APR 96
COPYRIGHT (C) 1996 DERWENT INFORMATION LTD

FILE 'CANCERLIT' ENTERED AT 16:19:13 ON 02 APR 96

FILE 'WPIDS' ENTERED AT 16:19:13 ON 02 APR 96
COPYRIGHT (C) 1996 DERWENT INFORMATION LTD

FILE 'DISSABS' ENTERED AT 16:19:13 ON 02 APR 96
COPYRIGHT (C) 1996 University Microfilms, Inc.

FILE 'CONFSCI' ENTERED AT 16:19:13 ON 02 APR 96
COPYRIGHT (C) 1996 Cambridge Scientific Abstracts (CSA)

FILE 'SCISEARCH' ENTERED AT 16:19:13 ON 02 APR 96
COPYRIGHT (C) 1996 Institute for Scientific Information (ISI) (R)

=> s ring d?/au
L189 93 FILE BIOSIS
L190 48 FILE MEDLINE
L191 44 FILE EMBASE
L192 38 FILE LIFESCI
L193 5 FILE BIOTECHDS
L194 27 FILE CANCERLIT
L195 28 FILE WPIDS
L196 6 FILE DISSABS
L197 12 FILE CONFSCI
L198 91 FILE SCISEARCH

TOTAL FOR ALL FILES
L199 392 RING D?/AU

```
=> s l199 and (fc? or cd16 or cd 16)
L200          7 FILE BIOSIS
L201          6 FILE MEDLINE
L202          7 FILE EMBASE
L203          3 FILE LIFESCI
L204          1 FILE BIOTECHDS
L205          9 FILE CANCERLIT
L206          1 FILE WPIDS
L207          0 FILE DISSABS
L208          1 FILE CONFSCI
L209          4 FILE SCISEARCH

TOTAL FOR ALL FILES
L210         39 L199 AND (FC? OR CD16 OR CD 16)

=> s l210 not (l55 or l101 or l77)
L211          3 FILE BIOSIS
L212          3 FILE MEDLINE
L213          4 FILE EMBASE
L214          2 FILE LIFESCI
L215          1 FILE BIOTECHDS
L216          4 FILE CANCERLIT
L217          0 FILE WPIDS
L218          0 FILE DISSABS
L219          1 FILE CONFSCI
L220          2 FILE SCISEARCH

TOTAL FOR ALL FILES
L221         20 L210 NOT (L55 OR L101 OR L77)

=> dup rem l221
PROCESSING COMPLETED FOR L221
L222           7 DUP REM L221 (13 DUPLICATES REMOVED)

=> d 1-7 bib abs; fil hom

L222 ANSWER 1 OF 7  BIOSIS  COPYRIGHT 1996 BIOSIS      DUPLICATE 1
AN  93:97264  BIOSIS
DN  BA95:52460
TI  IN-VITRO CYTOTOXIC TARGETING BY HUMAN MONONUCLEAR CELLS AND
    BISPECIFIC ANTIBODY 2B1 RECOGNIZING C-ERBB-2 PROTOONCOGENE PRODUCT
    AND FC-GAMMA RECEPTOR III.
AU  HSIEH-MA S T; EATON A M; SHI T; RING D B
CS  CHIRON CORPORATION, 4560 HORTON STREET, EMERYVILLE, CALIF. 94608.
SO  CANCER RES 52 (24). 1992.  6832-6839. CODEN: CNREA8 ISSN: 0008-5472
LA  English
AB  Bispecific murine monoclonal antibody 2B1, possessing dual
    specificity for the human c-erbB-2 protooncogene product and human
    Fc.gamma. receptor III (CD16) was evaluated for the
    ability to promote specific lysis of c-erbB-2-positive tumor cells in
    vitro. In short-term 51Cr release assays with human mononuclear cells
    as effectors and SK-Br-3 human breast cancer cells as targets,
    neither parental antibody of 2B1 mediated significant specific lysis,
```

but bispecific antibody was as active as a chemical heteroconjugate, with 5 ng/ml of 2B1 causing half-maximal lysis at an effector/target ratio of 20:1 and 2 ng/ml 2B1 causing half-maximal lysis at an E/T ratio of 40:1. The cytotoxic targeting activity of 2B1 F(ab')2 fragment was the same as that of whole bispecific antibody, and the activity of whole 2B1 was not reduced when assays were performed in 100% autologous human serum, indicating that 2B1 binds effector cells through the **CD16**-binding site derived from parental antibody 3G8 rather than through its **Fc** portion. Variable inhibition of 2B1-mediated lysis was observed when autologous polymorphonuclear leukocytes from different donors were added to mononuclear effector cells at a 2:1 ratio; this inhibition was overcome at higher antibody concentration. 2B1 bispecific monoclonal antibody was also able to mediate targeted cytolysis using whole human blood as a source of effector cells or using effector or target cells derived from ovarian cancer patients.

L222 ANSWER 2 OF 7 BIOSIS COPYRIGHT 1996 BIOSIS DUPLICATE 2
AN 91:478829 BIOSIS
DN BA92:112589
TI SELECTION OF HYBRID HYBRIDOMAS BY FLOW CYTOMETRY USING A NEW COMBINATION OF FLUORESCENT VITAL STAINS.
AU SHI T; EATON A M; **RING D B**
CS DEP. IMMUNOL., CETUS CORPORATION, 1400 53RD STREET, EMERYVILLE, CALIF. 94608.
SO J IMMUNOL METHODS 141 (2). 1991. 165-176. CODEN: JIMMBG ISSN: 0022-1759
LA English
AB A new combination of fluorescent dye (rhodamine 123 and hydroethidine) was used to internally label hybridoma fusion partners. Murine hybridoma 520C9 (recognizing human c-erbB-2) was labeled with hydroethidine. Murine hybridoma 3G8 (recoginizing human **Fc**.gamma. receptor III) was labeled with rhodamine 123, and verapamil was used to block rhodamine efflux of via P-glycoprotein. Viability assays showed little cytotoxicity from these dyes at the concentrations used. The labeled cells were fused with polyethylene glycol, sorted for dual flourescence on an Epics V cell sorter, and cloned. Hybrid hybridomas producing bispecific antibodies were selected for ability to promote lysis of SK-Br-3 breast cancer cells by human mononuclear cells. Several positive clones were obtained and shown to have a double content of DNA. Bispecific antibody produced by subclone 2B1 was purified by anion exchange chromatography and shown to bind both tumor cells and **Fc**.gamma.R III bearing cells. Using two parameter flow cytometric analysis, we were able to measure a 'bridging' effect of this bispecific antibody, which caused formation of complexes between PMNs and SK-Br-3 cells. Either parental antibody could compete with bispecific antibody to block such complexing. This function method provides several advantages over other techniques presently used (speed, convenience, low toxicity and automatic exclusion of dead cells) and can be applied to produce other hybrid hybridomas.

L222 ANSWER 3 OF 7 BIOTECHDS COPYRIGHT 1996 DERWENT INFORMATION LTD

AN 92-14294 BIOTECHDS
TI Targeting cytotoxicity with a bi-specific antibody directed to c-erb-2 and human **Fc**-gamma receptor III;
human oncoprotein human **Fc**-gamma receptor-III **CD16** bispecific antibody production, purification;
tetradoma 2B1 construction (conference paper)
AU **Ring D B**
CS Cetus
LO Department of Immunology, Cetus Corporation, 1400 Fifty-Third Street, Emeryville, CA 94608, USA.
SO Antibody Eng.; (1991) 3 pp.
DT Journal
LA English
AN 92-14294 BIOTECHDS
AB Hybrid hybridoma 2B1 producing a bispecific antibody with binding sites for human oncogene product c-erbB-2 (aka HER2 or neu) and for human **Fc**-gamma receptor-III (aka **CD16**) was prepared by fusion of hybridoma 520C9 (mouse IgG1-kappa recognizing the oncoproteins) and 3G8 (recognizing **Fc**-gamma receptor-III or **CD16**). The bispecific antibody was purified by DEAE-Sepharose, mono-Q-HPLC and SDS-PAGE anion-exchange chromatography in reasonable yield, since heterologous pairing of antibody light and heavy chains was not observed in this particular hybrid hybridoma. 2B1 bispecific antibody targeted human LGLs and cultured human monocytes to lyse a variety of c-erbB-2 positive human cancer cell lines. Cytological targeting was not diminished in 100% autologous human serum, and were observed in conditions designed to model an in vivo situation (e.g. in the presence of excess PMNs, in whole blood, or with effector and target cells from human cancer patients). Other bispecific antibodies formed from fusions of 3G8 are discussed, and include P-glycoprotein-specific and **Fc**-gamma receptor-III-specific antibodies produced by 1A7. (0 ref)

L222 ANSWER 4 OF 7 CANCERLIT
AN 92675551 CANCERLIT
TI TARGETED LYSIS OF HUMAN BREAST CANCER CELLS BY HUMAN EFFECTOR CELLS ARMED WITH BISPECIFIC ANTIBODY 2B1 (ANTI-C-ERBB-2/ANTI-**FC** GAMMA RECEPTOR III) (MEETING ABSTRACT).
AU **Ring D B**; Shi T; Hsieh-Ma S T; Reeder J; Eaton A; Flatgaard J
CS Dept. of Immunology, Cetus Corporation, 1400 53rd St., Emeryville, CA 94068
SO Non-serial, (1990). Fourth International Workshop on Monoclonal Antibodies and Breast Cancer. November 5-6, 1990, San Francisco, CA, 1990.
DT (MEETING ABSTRACT)
FS ICDB
LA English
EM 9112
AB Hybridomas 520C9 (murine IgG1 recognizing the proto-oncogene product c-erbB-2) and 3G8 (murine IgG1 recognizing human **Fc** gamma R III or **CD16**) were stained with vital fluorescent dyes

and fused with PEG; hybrid hybridomas were isolated by dual fluorescence on a cell sorter. Cultures whose supernatants promoted lysis of SK-Br-3 breast cancer cells by human total mononuclear cells were chosen for subcloning. The immunoglobulin species produced by clone 2B1 could be resolved on anion exchange chromatography into parental antibody 520C9, a middle peak containing bispecific antibody, and parental antibody 3G8. The bispecific peak further resolved into major and minor peaks on cation exchange chromatography. The major peak appeared bispecific on the basis of SDS PAGE, isoelectric focusing, immunofluorescent binding to both tumor and effector cells, and ability to promote targeted cytolysis. The minor peak also appeared bispecific by gel criteria, but lacked tumor cell binding and cytolytic targeting ability. Further analysis indicated that immunoglobulin from the minor peak was more heavily glycosylated than that from the major peak, and that Fab fragments released from the minor peak material regained tumor cell binding ability. We tentatively suggest that the minor species of bispecific antibody is more heavily glycosylated at the hinge region in a manner that sterically interferes with activity of the 520C9 but not the 3G8 binding site. No evidence for immunoglobulin species containing heterologous light/heavy chain pairing was observed. Purified 2B1 bispecific antibody (active form) promoted lysis of erbB-2 positive breast, ovarian and colorectal cancer cells by human LGLs and macrophages. Half maximal targeting activity was usually observed at antibody concentrations between 10 and 100 ng/ml. Target cell lysis was not blocked by the level of irrelevant immunoglobulin found in 100% autologous human serum. Lysis by human LGLs was somewhat reduced in the presence of high numbers of human PMNs, but substantial targeted killing due to 2B1 could be observed in unfractionated human blood.

L222 ANSWER 5 OF 7 BIOSIS COPYRIGHT 1996 BIOSIS DUPLICATE 3
AN 89:335407 BIOSIS
DN BA88:38407
TI EFFECTOR CHARACTERISTICS OF THE IGG-3 MURINE MONOCLONAL ANTIBODY 113F1.
AU WEINER L M; ZAROU C M; O'BRIEN J; **RING D**
CS DEP. MED. ONCOL., FOX CHASE CANCER CENT., CENTRAL AND SHELMIRE AVE., PHILADELPHIA, PA. 19111.
SO J BIOL RESPONSE MODIF 8 (3). 1989. 227-237. CODEN: JBRMDS ISSN: 0732-6580
LA English
AB Successful immunotherapy with unconjugated murine monoclonal antibodies (MAbs) is likely to require antibodies with potent effector characteristics such as antibody-dependent cellular cytotoxicity (ADCC). The newly developed IgG3 isotype antibody, 113F1, binds to a target antigen present in high copy number on SK-Br-3 cells surfaces. 113F1 does not appear to participate in complement-mediated cytotoxicity, but is a potent mediator of ADCC by human monocytes directed against a variety of malignant cells lines expressing 113F1's target antigen. Although recombinant interleukin-2 (rIL-2) promotes nonspecific monocyte cytotoxicity, preincubation with this cytokine has no effect on monocyte ADCC in our system.

113F1 promotes ADCC by non-adherent cells expressing lymphokine-activated killer (LAK) activity following incubation of these cells in rIL-2. These latter effects are abrogated by **Fc** receptor blockade prior to addition of 113F1. 113F1 is a more potent mediator of ADCC by monocytes or LAK cells than is 17-1A, an extensively tested unconjugated murine IgG2a isotope MAb. These data suggest that unconjugated 113F1 MAb immunotherapy trials should investigate treatment with the MAb alone. In addition, 113F1 could be administered with rIL-2, either alone or supplemented with infusions of autologous LAK cells.

L222 ANSWER 6 OF 7 EMBASE COPYRIGHT 1996 ELSEVIER SCI. B.V.DUPLICATE 4
AN 88269403 EMBASE
TI Targeted cytotoxic cells as a novel form of cancer immunotherapy.
AU Segal D.M.; Garrido M.A.; Perez P.; Titus J.A.; Winkler D.A.; **Ring D.B.**; Kaubisch A.; Wunderlich J.R.
CS Immunology Branch, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892, United States
SO MOL. IMMUNOL., (1988) 25/11 (1099-1103).
ISSN: 0161-5890 CODEN: IMCHAZ
CY United Kingdom
DT Journal
FS 016 Cancer
026 Immunology, Serology and Transplantation
LA English
AB Several classes of cytotoxic cells have been targeted using heteroconjugated antibodies. Antibodies against components of the T-cell receptor complex (either T(i) or CD2) (Perez et al., 1985; Staerz et al., 1985) or against CD2 (in man) (Siliciano et al., 1985) and Ly6 (in mouse) (Leo et al., 1987) redirect the specificity of cytotoxic T cells. Other types of cells have been targeted through **Fc**(.gamma.) receptors: human K-cells through the **Fc**(.gamma.)RIII (Titus et al., 1987b), also known as **CD16**, and monocytes and neutrophils through **Fc** (.gamma.)RI and II (Shen et al., 1986, 1987; Graziano and Fanger, 1987). Lysis mediated by targeted T- and K-cells is enhanced by incubating the effector cells with IL-2 (Titus et al., 1987b), whereas killing by monocytes and neutrophils is boosted by interferon-.gamma. (Graziano and Fanger, 1987). In this paper we will summarize our recent studies using targeted T- and K-cells to kill tumor cells, both in vitro and in vivo.

L222 ANSWER 7 OF 7 CONFSCI COPYRIGHT 1996 CSA
AN 91:52001 CONFSCI
DN 92020443
TI Targeting cytotoxicity with a bi-specific antibody directed to c-erbB-2 and human **Fc** gamma receptor III
AU **Ring, D.B.**
CS Dep. Immunol., Cetus Corp.
SO IBC USA Conferences Inc., 8 Pleasant St., Build. D, South Natick, MA 01760, USA. Telephone: (508) 650-4700. Fax: (508) 653-1627..
Meeting Info.: 914 5023: Second Annual IBC International Conference on Antibody Engineering (9145023). San Diego, CA (USA). 16-18 Dec